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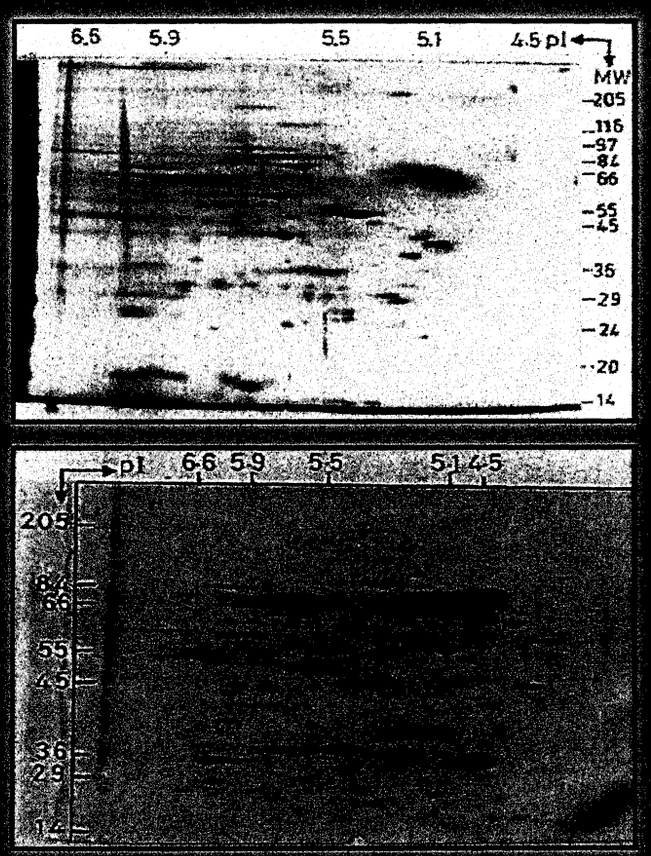

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EDITORS' PAGE

At the time of writing this Editors' Page, the first idea which came as a "flash" was to leave the page BLANK. Luckily, the idea was short-lived. However, this flash does raise a valid question. Why such a ludicrous idea should even come at all? Is it due to apathy of the colleagues (specially fellows of this Academy) towards the Academy journals. Possibly yes!

This academy has more than 1000 highly respected Fellows and almost equal number of Members and yet we are short of good research papers. Fellows hardly write for this journal which is their own Academy's journal. In this backdrop, will a blank "Editors' Page" serve as a reminder to the Fellows of the Academy to contribute at least one research paper in 3 years (a repeated request being made by us in the past) which would be enough to sustain the journal at a high scientific level? Probably not! This is a negative approach. We prefer a more positive and modest approach of "appeal" to all the Fellows/Members to play a proactive role in the affairs of the Academy, including the publication of this journal.

The official journal of this Academy viz., Proc. National Acad. Sciences in 1930-50 period had some international respectability because it was served by articles from science doyens of that time like Prof M.N. Saha, Prof N.R. Dhar, Prof A.C. Banerji and many others. The same was true for Proc. Indian Acad. of Sciences publishing contributions from Prof C.V. Raman, Prof S. Bhagvantam, Prof V. Sarabhai and many others. The present day Indian Scientists avoid publishing in Indian Journals, including Academy's journals, by using arguments like "impact parameter", international visibility, low circulation etc. These are valid points in the interest of individuals, particularly when, at each level of recognition by fellow Indian Scientists, emphasis is on the papers published in "foreign journals". The argument may be self-defeating in the long run. We have to strike a balance between improving Indian journals through our contributions to them and the compulsion of contributing articles only to the established foreign journals. How to begin? Where to begin? This "Catch-22" situation has been pointed out by Prof U.R. Rao (Nat. Acad. Sci. Lett. Vol. 27, 47-51, 2004). In such a situation, the "lead" has to come from senior scientists and fellows. It is upto them to respond to our sincere and modest appeal.

Let us hope for a marked improvement in the standard of Indian journals after adoption of a balanced approach by fellow Indian Scientists regarding their research publications. Are we wrong or are we expecting too much!!

Girjesh Govil
Jai Pal Mittal
Suresh Chandra

The views expressed here are solely those of one of the Editors and do not necessarily reflect those of the Academy or the Institute where he works.

Chronobiology: Implications of circadian rhythms

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Abstract

Biological rhythms are pervasive. All eukaryotes and at least one group of prokaryotes, the cyanobacteria, exhibit biological rhythms. Further, all levels of biological integration, such as ecosystem, population, group, individual, organ-system, organ, tissue, cell and subcellular structure show rhythms with diverse frequencies. Chronobiology (chronos - time; bios - life; logos - science) is the branch of science that deals with the study of biological rhythms and their mechanisms. It is multidisciplinary in nature and burrows techniques from many other disciplines. Circadian rhythms, popularly called biological clocks, are the most-studied mechanisms. They exhibit three important properties: 1) persist (or free-run) with a τ (tau) very close to 24 h in the absence of environmental time cues like day and night cycle; 2) entrain to 24-h cycles of environmental zeitgeber, most commonly the light/dark transition at dawn and dusk; and 3) possess temperature compensated free-running periods over a wide range of environmental temperatures, but within the physiological range. The basic circadian system consists of at least three interconnected components: 1) input pathways (photoreceptors, thermoreceptors etc.); 2) pacemaker (clock, such as SCN); and 3) effector pathways (overt rhythms). Molecular bases of cyanobacterial and eukaryotic circadian clocks have been partly understood with respect to their period length, sustainability, and relationship with light. The underlying molecular mechanisms of these clocks seem to have extraordinary degree of evolutionary conservation and appear to be a universal feature of the clocks in models as divergent as algae, fungi, fruit flies, mice and humans. This article outlines the concepts and current developments in the field of chronobiology with a reference to research on circadian rhythms. It also discusses the implications of circadian rhythms in the optimization of treatments in the clinics and in the management of problems in shift workers.

(Keywords : circadian rhythm/ clock genes/ chronotherapy/shift work)

Introduction

The rotation of the Earth on its axis causes the most pervasive periodic phenomenon, the day and night. There are many other periodic events in nature, namely the seasons, lunar cycle, tidal cycle, temperature cycle and the like, of which the cycle of day and night is the most dominant and predictable event. Microorganisms, plants, animals and humans exhibit biological rhythms, essentially similar in frequency to one of the geophysical

counterparts, in many overt behavioral, physiological, and molecular processes. All levels of biological integration: ecosystem, population, group, individual, organ-system, organ, tissue, cell and subcellular structure exhibit rhythms with different frequencies. Chronobiology is a relatively new branch of science that deals with the study of biological rhythms and their underlying mechanisms. It is multidisciplinary in nature and burrows techniques from many other disciplines to investigate biological rhythms in living organisms.

Historical outline

The credit for observing biological rhythm for the first time goes to a Greek philosopher cum an army officer, Androsthenes in the corps of Alexander the great (350 B.C.), who during the latter's march on India witnessed and made a note in his dairy that the leaves of *Tamarindus indica* exhibit sleep movement.[1]

J. C. Bose was the first Indian who performed elegant experiments and demonstrated biological rhythms in many plant species. He demonstrated diurnal variation of excitability in *Mimosa,* daily opening and closing of the leaflets in *Cassia alata* and diurnal rhythms in petal movements in water lily, *Nymphaea* and Jhinga, *Luffa acutangula.*[2] He demonstrated the phenomenon of entrainment of rhythms in various kinds of plant movements to alteration of light and darkness. Further, although Bose was not aware of it, some of the plants he was studying were actually expressing endogenous rhythms.[3]

In the real sense circadian rhythm research started in 1930s when Erwin Bünning and his colleagues discovered free running rhythm in leaf movement of the common bean *Phaseolus vulgaris.*[4, 5] He observed that the leaf movements of beans oscillated in constant darkness with a period of about 25.4 h and he was also the first to demonstrate that exposure to light/dark cycle of 24 h could entrain the 25.4 h free-running rhythm of *Phaseolus* leaf movement (reviewed by Sweeney[6]). This was the period when von Frisch[7] and von Frisch & Lindauer[8] observed the remarkable time sense in bees and Kramer[9] demonstrated that starlings used the sun as a compass to migrate. Hoffmann[10] further demonstrated that the clock persisted in constant dim light and thus is endogenous to the birds. Chronobiology witnessed major breakthroughs in the mid-twentieth century (1950-60). Pittendrigh[11] demonstrated the temperature compensatory behavior of circadian clocks. By then Bünning and Pittendrigh together discovered all three important diagnostic features of circadian clocks, which are valid even today. During this period chronobiology was accepted as an important quantitative biological science.[12] Now, it is well known that the biological rhythms have far-reaching implications. Undoubtedly, it is going to remain in the forefront of biomedical research in this century.

Types of rhythms

Rhythms, depending upon the periods they exhibit, are mainly classified into three types, viz., circadian, ultradian and infradian. Circadian (circa = approximately; dies = 24 h) rhythm relates to any biological variations with a frequency of 1 cycle per about 24 h. Ultradian rhythm exhibits a frequency ($\tau < 20$ h) higher than that of the circadian, whereas, infradian rhythm relates to certain biological variations that shows a frequency ($\tau > 28$ h) lower than that of the circadian.[13] One

may come across many other terms describing rhythms in organisms, namely circatidal rhythm or tidal rhythm, diurnal rhythm, diel rhythm, circalunar rhythm or lunar rhythm or circamensual rhythm or circasynodic rhythm in chronobiological literature. However, there is no reason to become confused by these terms. The tidal rhythm falls in the ultradian category. The lunar rhythm is synonymous with circatrigintan rhythm. Among all rhythms described above circadian rhythm is the most studied one. The term *circadian* was coined by Halberg[14] to replace the formerly used terms such as daily, diurnal, diel, 24-hour, and nycthemeral.

Thus, by definition biological rhythms that continue to persist with a period of about a day with all obvious environmental factors constant are called circadian rhythms.

Properties of circadian rhythms

All circadian rhythms have following properties in common[15]: 1) the rhythms oscillate with a period length close to, but seldom equal to 24 h, when organisms are kept under constant conditions of light, temperature and other possible geophysical factors, which could provide information about time to the organisms. Under these conditions the rhythm is said to be free running and its period is called free-running period (Fig.1); 2) the rhythms are entrained to 24-h cycles of environmental time cues, most commonly the light/dark transition at dawn and dusk. They may also get entrained to periodicity in other geophysical factors, namely temperature and humidity; and 3) the period of the free-running rhythm is temperature compensated within the physiological range. It has a Q_{10} close to 1, with observed values ranging from 0.8 to 1.3.

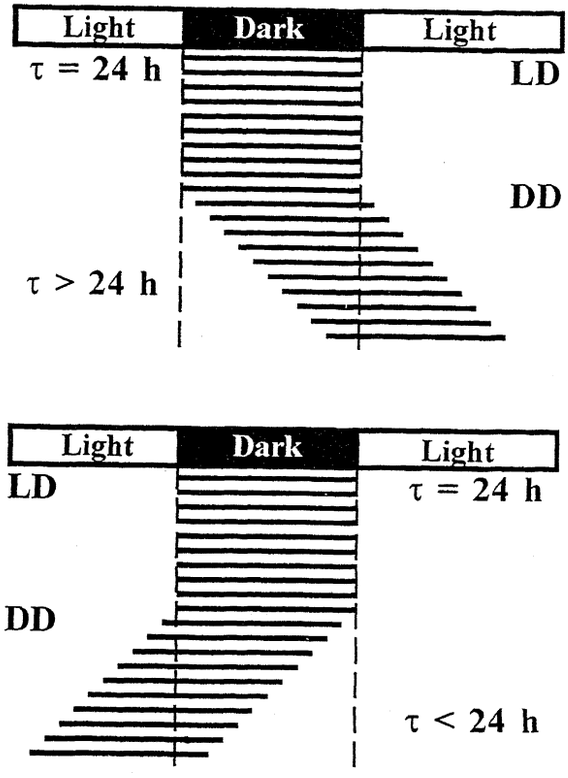

Fig. 1– Actograms showing idealized circadian rhythms immediately after a transfer from light-dark cycle (LD) to constat darkness. In the LD cycle the rhythm is entrained with the function (dark line) present only during the dark period. In DD the rhythm free-runs with the function onset occurring later (upper panel) or earlier (lower panel) each day. The successive 24-h records are arranged one above the other.

Interestingly, the circadian rhythms may also differ from one another in a number of ways: 1) one rhythm may be more sensitive to light, while others are less; 2) the patterns of the phase response curves (PRCs) may vary; 3) some rhythms are reentrained quickly, while others may take several days before achieving a stable reentrainment; 4) some rhythms damp quickly, while others persist for several cycles in a constant environment; 5) when

rhythms persist in continuous light, the characteristics of the rhythm (period and PRC) may vary with the colour and/or intensity of the background illumination.[15]

Components of the circadian system

Circadian rhythms are endogenous. They are both an organismal and a cellular phenomenon.[16] Thus a basic circadian system has necessarily three important components, such as photoreceptors, pacemaker(s), and observable rhythmic outputs (Fig. 2). The entraining pathways transduce information between the photoreceptive elements and the pacemaker(s). The coupling pathways are also necessary as a link (the efferent pathways) between the pacemaker(s) and the multiple

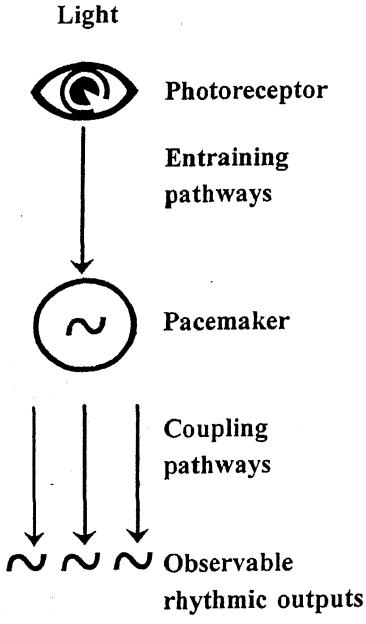

Fig. 2–A model showing a simple unidirectional pathway consisting of basic components of circadian systems.

effector systems. The effector outputs are the overt rhythms that allow us to study the properties of the central clockwork (pacemaker). There may be multiple photoreceptors, multiple clocks and many overt rhythms (Fig. 3). These components may interact with each other in a variety of ways. A possible operation of feedback of the clock onto the photoreceptive pathway has been strongly suspected.[17]

Location of the clocks

In the past, several attempts have been made to locate the endogenous circadian clock in the animal body by performing lesion experiments with the presumption that the destruction of the pacemaker would result in the disappearance or desynchronization of the overt rhythms. Such attempts have been made in diverse animal models, such as cockroaches, crickets, moths, crayfish, crabs, molluscs, reptiles, amphibians, fish, birds and mammals.[18]

In most of the animals the master clock is located in the central nervous system. Kawamura & Ibuka[18] localized the circadian clocks in the optic lobe of cockroach and cricket. In lower vertebrates, experimental evidence suggested that the pineal and retina contain endogenous pacemakers.[19] In higher plants, populations of pulvinar and guard cell protoplasts have been shown to function as endogenous clocks.[16] The retinal clocks have also been demonstrated in mammals. [19, 20] The master circadian clock of mammals resides in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus.[21] It has been documented that SCN has a number of single-cell circadian oscillators and in entrained state these oscillators regulate overt circadian rhythms through the generation of coordinated circadian outputs.

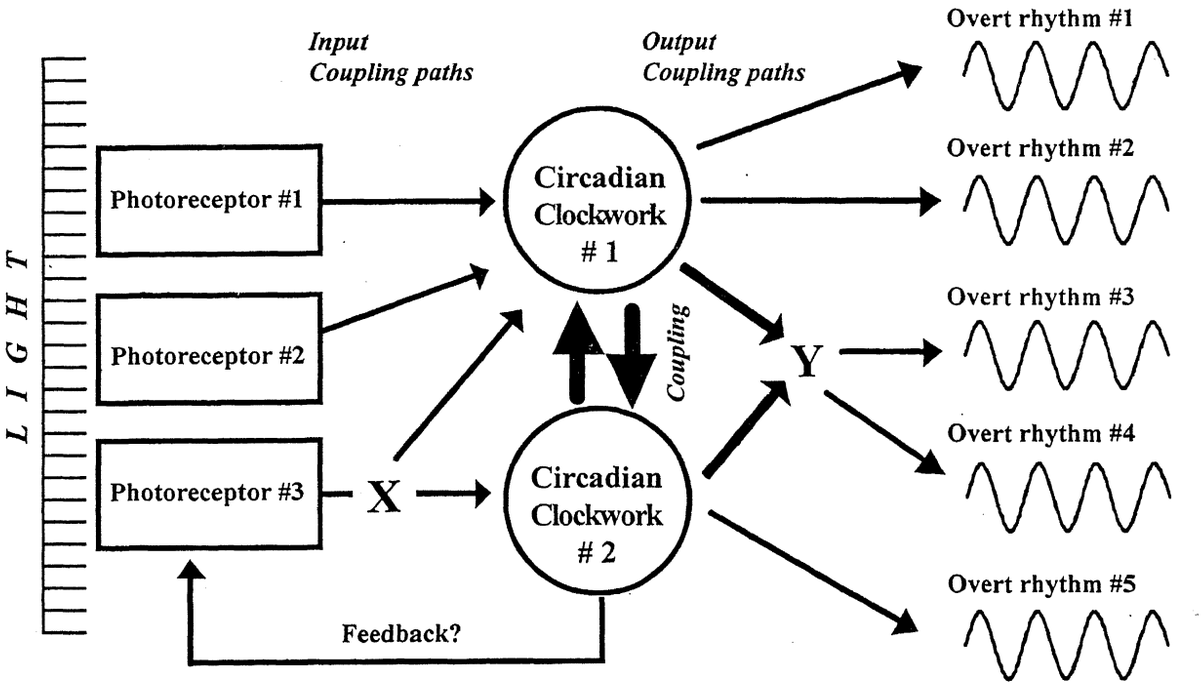

Fig. 3– A hypothetical model for the organization of the major components of circadian systems. Light information is perceived by photoreceptors that pass the information along central clockworks by input coupling pathways. The clockworks are circadian oscillators that are phased by input pathways and control via output coupling paths various overt rhythms (outputs). The multiple inputs, clockworks, and outputs may be coupled in a variety of ways, including components that might be common to more than one input (e.g. X) or output (e.g. Y) pathway (Modified from Johnson[17]).

Peripheral clocks

It has been presumed that the mammalian circadian timing system is composed of almost as many individual clocks as there are cells.[22] However, temporally coordinated physiology can be achieved only when these countless clocks remain in a state of meaningful synchronization. In mammals, the master clock, the hypothalamic SCN, executes this job. In addition, feeding time has been established as a dominant cue for the oscillators in several peripheral tissues. It has also been speculated that the locomotor activity rhythm can feed back on the SCN by modifying period length and phase.[22] The mechanisms by which peripheral clocks remain coupled/uncoupled from the central pacemaker are yet to be understood completely. The peripheral oscillators in *Drosophila* and zebrafish can be directly entrained by light, but in mammals these clocks remain unresponsive to LD cycles.[23]

Molecular components of circadian clock

The discovery of X-chromosome-linked *period* (*per*) mutations in *Drosophila* by Konopka & Benzer[24] has laid the foundation of molecular chronobiology. The period gene appeared to possess three mutant alleles, namely long period gene (per^{l}), short period gene (per^{s}) and an abolish-period gene (per^{0}). The per^{l} and per^{s} mutants were found to have lengthened

(τ = 29 h) and shortened (τ = 19 h) circadian rhythms, respectively. The *per*^{0} mutants did not exhibit circadian rhythms in locomotor activity. The importance of *per* gene in the circadian clock of *Drosophila* has led to a series of interesting studies aimed at understanding clock genes in a range of other systems, such as cyanobacteria, green algae, fungi, higher plants and mammals. These studies conducted in the last couple of decades have partly unravelled the molecular basis of circadian clocks.[17, 21, 25-27]

The *per* gene has sequence homology with the *single-minded* (*sim*) gene, *ARNT* (human aryl hydrocarbon receptor nuclear translocator) gene in the *Drosophila* genome. All these genes share a common PAS (PER, ARNAT, SIM) protein domain.[28] This domain has been implicated in the protein-protein interaction. The PER protein binds with the protein product of another clock gene *tim (timeless)*. Both PER and TIM have been shown to be involved in the circadian systems of *Drosophila*.

Recent developments in the field of molecular chronobiology revealed two common features among clocks in all organisms.[25] Firstly, all clocks belonging to diverse systems are intimately associated with their photoreceptors. Secondly, the presence of PAS domain as one of the critical components of all clocks made up of a transcriptional feedback loop (Fig. 4). The clocks may differ from one another in two different ways: 1) the molecular mechanisms of photoreception may be different in different systems, and 2) the highly conserved transcriptional-translational-feedback loop of the clocks in microorganisms, plants, insects, and mammals may consist of different components and may require different types of molecular interaction between these components to generate the feedback loop.[25]

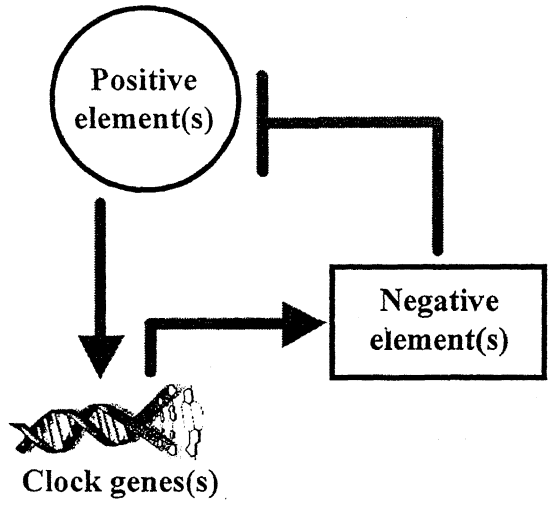

Fig. 4– A model showing a transcriptional feedback loop. Clock genes form this loop that negatively feeds back on their own transcription.

Characteristics of clock component

A clock component must fulfill three important conditions: 1) the activity or the amount of the component should oscillate; 2) complete loss of the component must lead to arrhythmicity; and 3) a phase shift in the clock should follow a transient change in the level of the component.[29, 30]

Clock genes in *Drosophila* and mammals

In *Drosophila* several clock genes have been identified. These include *period* (*per*), *timeless* (*tim*). *clock* (*clk*), *cycle* (*cyc*), *doubletime* (*dbt*), *cryptochrome* (*cry*), and *virile* (*vrl*). One or more mammalian homologs of these *Drosophila* genes have also been discovered. The mammalian genes include *Per1*, *Per2*, *Per3*, *Tim*,

Clock, *Bmal1*, *Tau*, *Cry1*, *Cry2*, and *E4bp4*.[31]

Toh et al.[32] reported that a mutation in *hPer2* is responsible for familial advanced sleep-phase syndrome (FASPS) in members of a Utah family. They found out that this gene is homologous to *Drosophila* and mouse genes those when mutated were known to speed up circadian rhythm. It was further demonstrated that the mutation disrupted phosphorylation of the hPER2 protein by an enzyme, casein kinase one-epsilon.[32]

The recent upsurge in the techniques in molecular biology may eventually help us in answering some of the basic questions related to the enigmatic circadian clock and its evolutionary significance. Nevertheless we know that the implications of circadian rhythms are many fold. Efforts are being made to use circadian principles to tackle issues related to human wellbeing.

Medical implications

Chronotherapy is a clinical strategy that harmonizes medical treatment with the patient's biological rhythms to have the maximum desired and the least undesired effects. It is based on the principle that the organisms respond differently to the same treatment modality or dose, directed against a specified ailment or disease, when administered at different points across any time scale, such as day, week, month and year. In other words, organisms, including human beings, exhibit rhythms of varying frequencies in susceptibility or sensitivity to a drug or any other kinds of treatment.

Indian physicians had the knowledge of time and its significance in the physiology and treatment of human diseases as early as 2500 B.C.[1, 33] However, rigorous scientific analysis of this concept started only in the middle of 20th century. Pohle et al.[34] for the first time documented that the dosing time of a carcinostatic drug may have a bearing on its antitumor activity. Since then chronobiologists have been reporting interesting time-related medical observations. However, chronobiology is still in the process of being accepted by the medical community. In recent years medical practitioners have started taking biological rhythms into account in diagnosing and treating diseases.

Preclinical chronopharmacologic studies

Chronopharmacologic studies first begun with the advent of new carcinostatic drugs. It is well known that most of the anticancer agents, such as oxaliplatin, carboplatin, doxorubicin, 4'-*O*-tetrahydropyranyladriamycin (THP), cisplatin, methotrexate, 5-fluorouracil, peptichemio, etoposide, vinblastine, vincristine, docetaxel, produce excessive host toxicity. A fundamental question arises: does the extent of host toxicity vary as a function of dosing time? Results from several studies were startling and revealed that host toxicity may be different depending upon the time of drug treatment on a specified time scale.[35, 36] The survival (end point of drug toxicity) in healthy rodents varies considerably, sometimes 3-fold and even more as a function of dosing time of several anticancer agents.[36] Now it has been well documented that the phase of murine chronotolerance is different for different drugs across a circadian time scale. The extent of toxicity and anticancer activity of over 30 anticancer agents that include anthracyclines, platinum analogs, alkylating agents, antimetabolites, spindle poisons, plant alkaloids and radiation have already been examined in mice or rats kept under controlled conditions of photoperiod

and temperature.[37, 38] The anticancer effect of these drugs was found to be circadian phase dependent. Thus the time of drug administration is critical with regard to chronooptimization.

In hepatic tissue of rodents, circadian changes in DNA synthesis, RNA synthesis, RNA translational activity, mitotic index, weight, glycogen content and activity of several enzymes are well known.[39, 40] The circadian rhythms in DNA synthesis in other tissues, such as stomach, duodenum, rectum and bone marrow, have also been documented in rodents.[41] These rhythms in all probability affect the drug pharmacokinetics leading to manifestation of circadian dependent chronergy. In addition, several drug-metabolizing enzymes in kidney also exhibit circadian rhythms in their activities. Physiological rhythms in cytokinetics, nucleic acid metabolism, immunological variables, drug metabolism, and hormones could serve as basis for time-dependent drug response of the organism.[40]

Chronopharmacology of cyclosporine-A

The immune system is indispensable for the organism's life, because it helps to prevent diseases by microorganisms, foreign molecules and malignant transformed cells. Several steps are necessary to make the immune system able to eliminate foreign agents. Firstly a foreign substance is identified and subsequently it is destroyed. In between these two steps many other important steps such as recognition, activation, proliferation and differentiation are involved. All these steps are auto-regulated and have been proposed in one of our reviews[42] to be coordinated along the circadian time scale both in mice and man (Fig. 5).

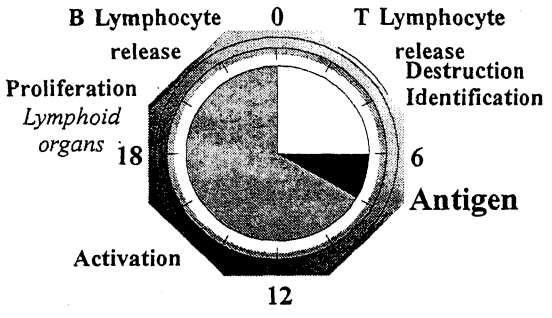

Fig. 5– Immune clock showing temporal coordination of different stages of immune defences. (Modified from Lévi *et al*[42]).

Cyclosporine-A (CiA) also known as ciclosporine (Cs) is a powerful immunosuppressive agent that prevents rejection of both experimental and clinical allografts. It is used in kidney, liver and heart transplantation, and bone marrow allografts. It seems to block the activation of resting T-cells. However, the renal toxicity of CiA constitutes its main dose-limiting side effects. The effects of the oral administration of different dosages of CiA have been examined on histological pictures of kidney in male B6D2F1 mice.[43] It was observed that CiA is nephrotoxic and the extent of toxicity varies as a function of its ingestion on a circadian time scale.[44] In another study attempts were made to determine the optimum time of oral administration of CiA by using 315 male B6D2F1 mice. Both toxicity and T-cell suppression were assessed.[44] It was observed that a satisfactory compromise between the least renal toxicity (Fig. 6) and the maximum immunosuppression could be achieved when CiA is given orally near the transition of dark (activity) to light (rest). The time dependent immunomodulation observed in this study could be attributed to a circadian-adapted immune surveillance mechanism.[42] Extrapolation of results from

these and other preclinical chronopharmacological studies involving beta-receptor blocking drugs, anesthetics, nonsteroid anti-inflammatory drugs, anticoagulants, and hormones has laid the foundation of chronotherapy in the clinics.

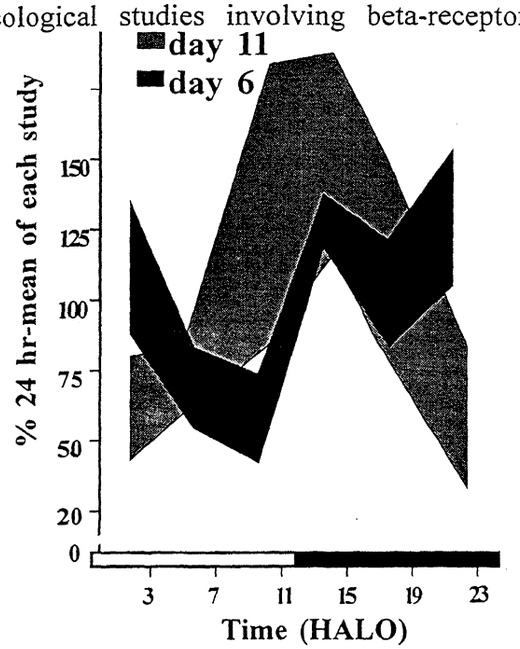

Fig. 6– Dosing-time dependent renal toxicity of CiA in mice, as gauged from the extent of microscopic renal lesions. Mice were killed 24 h after the 5th or 10th daily dose (100 mg/kg/day P.O.). Murine chronotolerance for CiA kidney lesions in a total of 105 male B6D2F1 mice receiving 100 mg/kg/day of CiA for 5 or 10 days; histologic lesions scored from 0 (normal) to 5 (extensive tubular necrosis). Their kidneys were removed and fixed in Bouin's picroformol solution for 24 h before being processed for histologic analysis. Hematein-eosine-stained slides were observed in random order by a histopathologist, who was unaware of treatment group. Tubular lesions were scored from 0 (no lesion) to 5 (necrotic spread throughout the whole cortex). Double readings performed several months apart yielded the same score in 60% slides, and differed by one unit in 38% of them. Highest renal toxicity corresponded to CiA ingestion at 15 HALO. Partial recovery of renal lesions were observed following CiA treatment at 23 or at 3 HALO. HALO = *H*ours *A*fter *L*ight *O*nset. The area shown within two lines defines the limits of CiA toxicity (Mean ± 1 SE) as a function of dosing time. (Based on data from Pati *et al.*[44]).

Chronotherapy in clinics

Currently chronotherapy is practiced in the clinics for a number of human diseases, notably cancer, allergy, asthma, arthritis, heart disorders, delayed and advanced sleep phase syndrome (DSPS and ASPS) and seasonal affective disorder.[38, 45-49]

Drug delivery system

Programmable-in-time, bedside, implantable and ambulatory pumps popularly called chronopumps are now available in the market. Drug can be delivered as multistep doses or as *on-off* timed boluses by using these pumps.[36] With regard to infusion modes, several possibilities exist for employing chronotherapy in managing human diseases involving analgesics, carcinostatics, and antihypertensive drugs. These pumps help in timing medications or in modulating the drug infusion rate along the circadian time scale in patients.[40, 50] Thus these pumps are used as tools to obtain reliable and desired chronotherapy.

Shift work

Shift work is indispensable in health care, security, transportation, manufacturing, and many other essential sectors that operate round the clock. Therefore, in these sectors groups or crews of workers succeed each other at the same workstations in shifts. Shift work disrupts biological rhythms, sleep and social life of workers. They suffer from a number of clinical and non-clinical problems.[51] Shift work retards human performance and

increases the chance for the occurrence of major industrial accidents.

Consequences of shift work

Disruption of circadian rhythms

Rotational shift work affects human health and performance by disrupting biological rhythms and by causing numerous alterations in their behaviour and physiology. The phenomenon of internal desynchronization is of commonplace among shift workers.[51,52] The desynchronization includes alterations in one or all of the important rhythm parameters, such as phase (peak), amplitude and 24-hour average. Pati & Gupta[53] reported disruption of circadian rhythm of time estimates (time intervals of 10-sec duration) in shift workers (Fig 7).

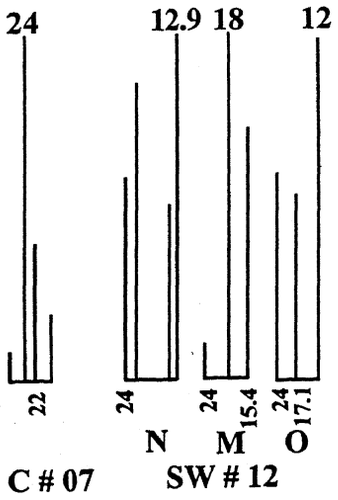

Fig. 7–Disruption of circadian rhythm of time estimation in shift worker. Power spectra of time estimation rhythm in a day worker (C # 07) and a shift worker (SW # 12). The prominent periods are expressed in h. In day worker prominent τ is 24 h, whereas τ differs from 24 h in the shift worker irrespective of the shifts (N = Night shift; M = Morning shift; O = Off days). (Based on data from Pati & Gupta[53]).

Consequences on sleep

Night shift workers suffer more often from insomnia like sleep disorder. This abnormality is characterized by difficulty in falling and staying asleep. The association between shift work and sleep disruption results in adverse medical and psychological consequences.[54, 55] In many studies, a majority of shift workers admit to having experienced involuntary sleep on the night shift, whereas this is rare on day-oriented shifts.[56, 57]

Several investigators have documented a significant circadian rhythm in subjective drowsiness/sleepiness in apparently healthy human subjects.[58-60] In these subjects, the drowsiness rhythm exhibits a peak between 21.0 to 23.0 h with a pronounced circadian period. However, in case of shift workers, rhythm in drowsiness desynchronizes externally as well as internally.[58, 59] Shift workers do have problems with sleep management specially because they attempt to have sleep at chronobiologically unsuitable time of the day. The problems include difficulty in initiating sleep and staying asleep. According to Czeisler et al.[61] initiation of sleep is very difficult at the acrophase (maximum) of the body temperature rhythm and very easy at its nadir (minimum). Shift work disrupts the normal relation between rest/activity rhythm and the circadian regulation of bodily functions.[62]

Social problems

The shift workers have been shown to experience a number of psychological disturbances and family dysfunctions, as a result of which there is a serious impact on the family and social life.[63-65] The irregular

work hours affect the whole family: the worker, his/her spouse and children.

Pati and Chandrawanshi[66] examined the effects of three-shift work schedules of shift workers on anxiety and mental health of their day active spouses and children. The levels of anxiety were found to be significantly higher in spouses and children of shift workers as compared with their counterparts sampled in the family of day workers. Also the status of mental health was significantly low among spouses of shift workers as compared with their day working counterparts.[66] This indicates that disturbed daily schedules of shift workers may modulate anxiety and mental health in their spouses and children.

Clinical problems

Shift work can lead to a host of problems attributed to the disturbances of the circadian system. It has been argued that shift work may result in significant morbidity. The long-term effects of shift work may induce coronary heart disease (CHD) and gastrointestinal diseases (see review Knutsson [67]).

Non-clinical problems

Performance

Poor sleep quantity (sleep deprivation) and quality have been considered as the key factors in modulating the performance of shift workers during the night shift.[68, 69] Furthermore, in shift workers sleep deprivation and desynchronization of biologic rhythms lead to impaired physical performance.[65, 70] Performance decrement has been reported in nurses during the night shift although there has been *no sleep deprivation.*[71] Thus these findings negate the hypothesis that implicates sleep deprivation or sleep debt as one of the major reasons for performance decrement.[71, 72] Could it be that sleep during the habitual timing, but not the length of sleep is imperative for normal human performance?

The circadian rhythm and sleep wake cycle are mainly related to the psychophysiology of shift work. People working either in rotating shifts or in a static/shift system have to work during the night at the low phase of their circadian rhythm. This leads to severe sleepiness and reduced performance.[63] The results of the studies conducted by Gupta[72] and Gupta and Pati[59] indicate that the shift rotation pattern is also important for normal performance. Studies on performance of shift workers working in three different type of rotational patterns revealed that 12-h night shift system for 15 consecutive days was the worst one as compared to other two shift patterns, i.e., 12-h night shift for 1 week and 8-h weekly rotational shift system.[59, 72]

Level of work performance efficiency on a night shift depends primarily upon: 1) the demands of task; 2) the type of shift system and hence potential for both short and long term adjustment; 3) individual differences between shift workers in the degree to which their rhythms adjust to night work, and 4) sleep deprivation.[54, 59, 73]

On duty accidents

Several studies have documented that accidents and injuries are imputed to sleep deprivation and disruption of sleep-wakefulness rhythm that occur on account of shift work.[65, 74] A number of studies have demonstrated that the rate of serious accidents is higher at night than during the day.[75-78] Furthermore, it has been observed that despite considerable reduced traffic

during night, single vehicle accidents occur past-midnight at a significantly higher rate.[79-81] Studies conducted on train drivers also revealed that they tend to overlook and/or issue more warning signals during the night shift. Various kinds of industrial injuries have also been shown to be 2-3 times higher during the night shift as compared with the evening shift.[82-84]

The first "chronotoxic" industrial disaster occurred at Bhopal.[85] Another important point is that the circadian risk of accidents was used to be considered from the individual point of view before Bhopal and Chernobyl disasters. Now the question is: how to prevent population disasters with high-risk industries (e.g. nuclear power plant, oil refinery). The peak time of risk at night involves not only few given night workers but the population dwelling around the plant.

Optimization of human shift work

A modern society probably cannot afford to abandon shift work, although it has been unequivocally established that it produces a series of acute and chronic effects on human beings. Therefore, there is an absolute need for an optimization of human shift work. Then question arises: how can the circadian rhythm desynchronization be minimized?

On the basis of studies done by our group and others it is suggested that each and every work places where shift work is mandatory, a chronoclinic should be established. Trained health care personnel of the chronoclinic should monitor intermittently (preferably every alternate year) the state of the biological clock (synchronized or desynchronized?) of each shift worker. Upon discovering rhythm desynchronization his/her transfer from shift work to day work for at least one year should be recommended to the employer/ management. This would perhaps rule out the possibilities of ill effects of shift work. It has been proposed that while examining tolerance/intolerance of a shift worker to rotational shift works, the levels of anxiety and mental health status of the individual under scrutiny should be taken into consideration. Sleep-wake disorder is another important variable that cannot be simply ignored while ascertaining intolerance to shift work. Appropriate chronotherapy, in addition, should also be administered into intolerant shift workers while they are being transferred from shift duty to day duty. A model has been proposed with a view to optimize shift work (Fig. 8). This model takes into account most of the important variables those are thought to have a bearing on the effective management of shift work.

Conclusions and future directions

The recent upsurge in the techniques in molecular biology may eventually help us in answering some of the basic questions related to the enigmatic circadian clock and its evolutionary significance. Our knowledge is fragmentary regarding relationship of the clocks with the non-photic stimuli. We also know very less about the peripheral clocks and their relationship with the central pacemaker. Nevertheless we know that the medical implications of circadian rhythms are many fold. In future circadian clocks would receive increased attention with reference to application of their principles in chronotherapy, in the management of problems in shift workers, and in the treatment of human sleep disorders. In addition, attempts should also be made to elucidate the molecular mechanisms of

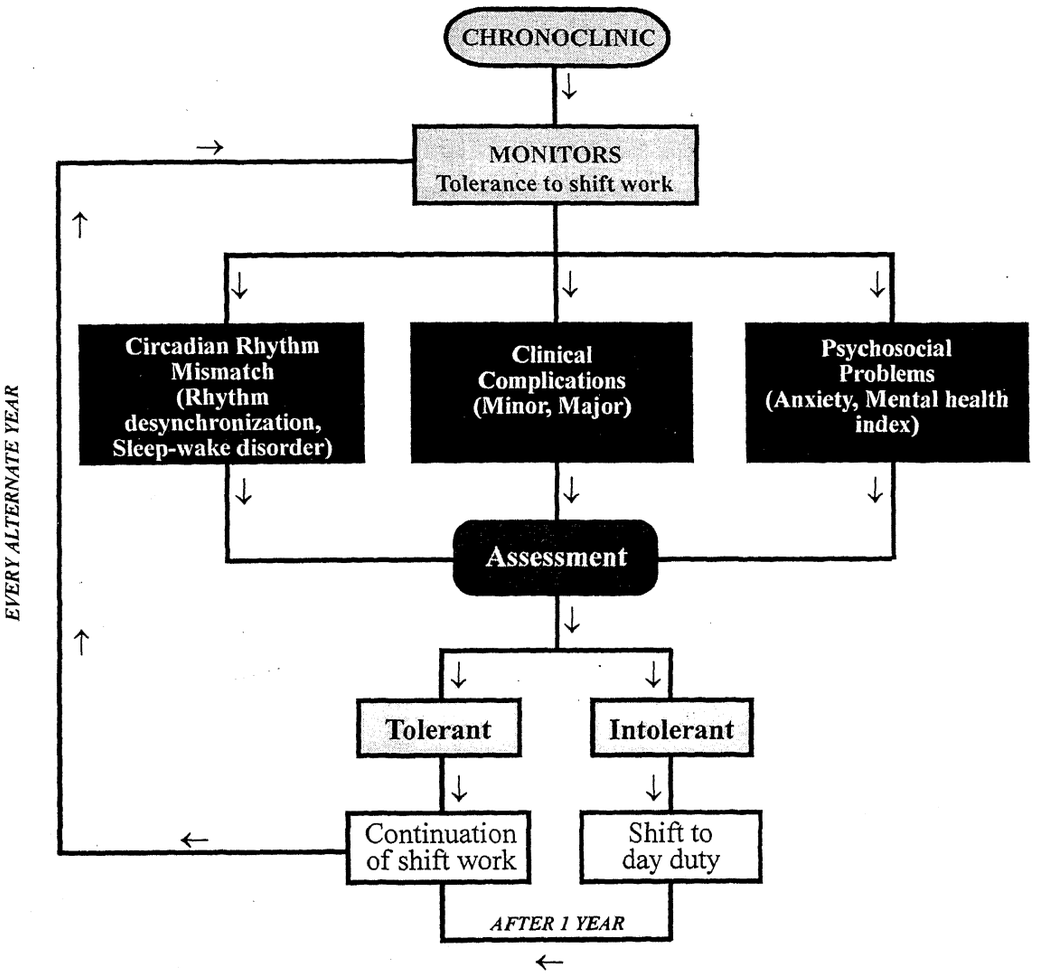

Fig. 8– Model suggesting optimization of human shift work. See text for details. (Modified from Pati *et al.*[51]).

noncircadian-based clocks, namely ultradian and infradian clocks and their relationship with the circadian clocks.

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Proteomics and human proteome organization

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"DNA makes RNA, RNA makes protein, and proteins make us." Francis Crick

Abstract

With complete genome sequences now available for several prokaryotic and eukaryotic organisms, biological researchers are faced with the unprecedented scientific challenges of assigning molecular and cellular functions to thousands of newly predicted gene products and explaining how these products cooperate in complex physiological processes. The field of proteomics, study of all the expressed proteins of an organism, has emerged with the goals of developing and applying methods for the global analysis of protein expression and function. Proteins determine the biological phenotype of an organism and are the primary targets for most therapeutic agents. HUPO (Human Proteomic Organization), an international coordinating group has been launched in 2001. This is a loosely knit federation of proteome researchers. HUPO helps set priorities, coordinate research, set standards for handling and processing samples, and arrange for the use of common bioinformatics to ensure that researchers can directly compare their results. The present paper highlights the recent human proteomic initiatives taken up by HUPO and the boundless future of proteomics in better management of human health and disease.

(Keywords : proteomics/MALDI-MS/HUPO/2D-gel electrophoresis)

Introduction

With complete genome sequences now available for several prokaryotic and eukaryotic organisms, biological researchers are faced with the unprecedented scientific challenges of assigning molecular and cellular functions to thousands of newly predicted gene products and explaining how these products cooperate in complex physiological processes. To address this problem, the field of proteomics has emerged with the goals of developing and applying methods for the global analysis of protein expression and function[1]. Proteomics is the study of all the expressed proteins of an organism[2]. The term 'proteome' or 'proteomics', first introduced in 1995[3], denotes the protein complement of the genome. The information that proteomics studies can provide includes expression levels, post-translational modifications, subcellular localization, protein-protein interactions, and protein-nucleic

acid interactions[2]. Proteomics has come a long way in a few years and this area of research is now a discipline in its own right (see for a series of reviews on different aspects of Proteomics : *Pro–teomics : A Trends Guide*; Blackstock, W. and Mann, M. (eds) Elsevier Science, 2000).

In the flow of genetic information from sequence to function, the stored information is translated twice, first from DNA to mRNA in the process of transcription, then from mRNA to protein in the process of translation. DNA and protein sequence comparisons have become routine steps in biochemical characterization, from newly cloned protein to ensign genomes. Genomics attempts to make a complete inventory of genes and nucleic acid sequences; in contrast to genomics approach, proteomics attempts to study the expressed protein. In fact, Proteome analysis supplements gene sequence data with protein information about where and in which ratio and under what condition proteins are expressed. Thus genomics and proteomics are complementary tools to study life sciences[4-8] (Fig 1).

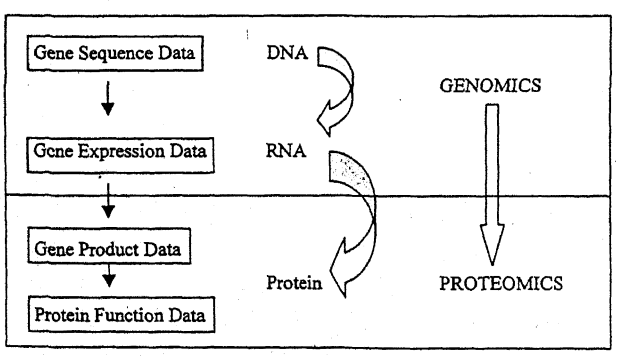


Fig 1– Genomics and Proteomics are synergetic (Adopted from Kellner 2000)

Proteomics Technology

The outline of a stereotypical proteome analysis is well established: (ideally) unbiased recovery of all protein constituents; fractionation of the protein mixture by two-dimensional polyacrylamide gel electrophoresis (2-D PAGE); generation of structural data by MS (usually following enzymatic hydrolysis of the proteins); and matching of analytical data against those predicted for constituents of a database of known proteins or anticipated expression products[9] (Fig 2).

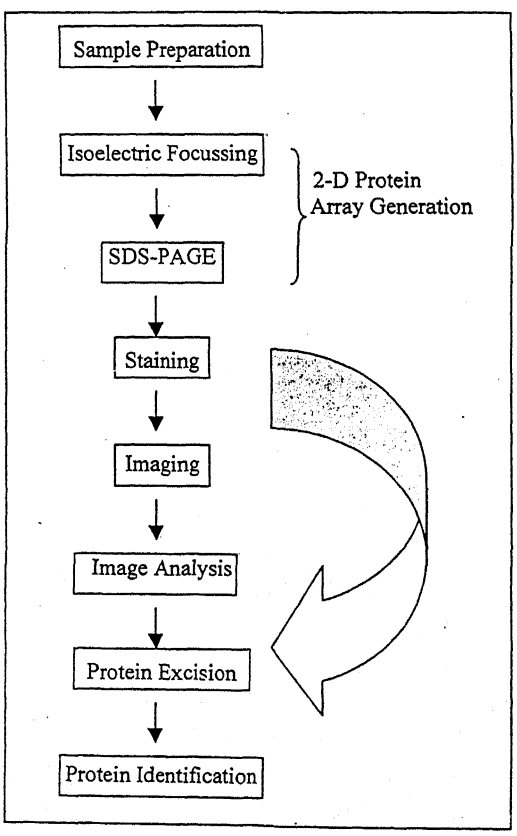


Fig 2– Steps in Proteomics

Two-dimensional polyacrylamide gel electrophoresis

2-D PAGE is a multi-step separation technique in which proteins are solubilized and separated according to charge (pI) in the first dimension using IEF, followed by size (molecular weight, MW) using SDS-PAGE in the second dimension[10]. Because the pI and the size of proteins are independent characteristics, one achieves excellent resolution of complex protein mixtures. Standardization and automatization of 2-DE is very important for confident assignment of even low intensity spots in areas of the gel with low spot density. 2-D based methods require the largest amount of tissue, often milligram quantities, but they return the largest amount of biologically relevant information about the diversity of the proteome[11]. The data collected in the 2-DE database are a source for basic research in Biochemistry & Biotechnology to build up a system of proteins, in analogy to the construction of the periodic system of elements in Chemistry and the construction of a system of living organisms by Linne [12-16].

Until now protein expression profiling using 2D-PAGE- based separation has provided the best 'snapshot of the protein repertoire of the cell or body fluid. However this technology is limited in that it is low throughput, labour-intensive and time consuming and also has problems detecting proteins that are basic in charge or smaller than 10 kDa. New mass spectroscopy- based multi dimensional separation systems with differential isotope tagging display technology do not have this specific limitation and are complementary to 2D- PAGE analysis. Unlike 2D-PAGE, isotope tagging can identify differentially expressed proteins regardless of their intrinsic hydrophobicity; can detect proteins in the lower molecular weight (attomole) range (in which 2D-PAGE does not provide good resolution for detection)[17].

A range of techniques has been developed to improve resolution and reproducibility. The separated proteins are stained with Coomassie or silver stain to produce a two-dimensional protein reference map (Fig 3)[18]. The 2-D gels are digitized and then curated using image analysis software to remove horizontal and vertical streaking and background haze. Replicate gel images can be aligned and matched with one another to generate a synthetic composite image. This is an important step because the proteome is a dynamic situation and captures biological variation as it occurs, and even 'orphan' proteins (proteins that do not appear on every gel) are included in the analysis. Gel sets showing protein maps from samples with different genetic, developmental or physiological backgrounds, can then be investigated globally for qualitative and quantitative differences[14].

Mass spectrometry/ Matrix-assisted laser desorption-ionization- mass spectrometry, MS/ MALDI MS

Mass Spectrometry (MS) encompasses a family of methods used to obtain accurate mass of ions in the gas phase. The components common to all mass spectrometers include an ion source, a mass analyzer and a detector, as well as a mechanism for recording and processing the mass spectra. MALDI-MS can profile the peptides and proteins from single-cell and small tissue samples without the need for extensive sample preparation, except for the cell isolation and matrix application[19]. In a mass spectrometer, molecules of a

compound are ionized, either by ejection of an electron or capture of a proton, to give the parent molecular ion, the energy of which is such that some fragmentation occurs to give a series of fragment ions.

Knowledge of the mass of the molecular ion and its major fragment ions is frequently sufficient to enable the structure of the parent compound to be uniquely deduced. The method is very sensitive and uses as little as 10^{-6} to 10^{-9}g of material. A mass spectrum is a plot of the abundance of the fragment and molecular ions against mass.

Protein Identification

Several approaches have been used to identify the proteins from 2-DE gels, but most rely on comparisons with sequence databases derived from genomic programmes, cDNA studies, protein sequencing or more recently from expression sequence tags (ESTs) and genomic sequence tags (GSTs) [20,21]. This is possible because of increased information available in databases as a result of rapid sequence technologies. Methods for the quantitation of relative protein abundance at the protein level are getting more advanced, which should complement gene expression monitoring at the mRNA level[8].

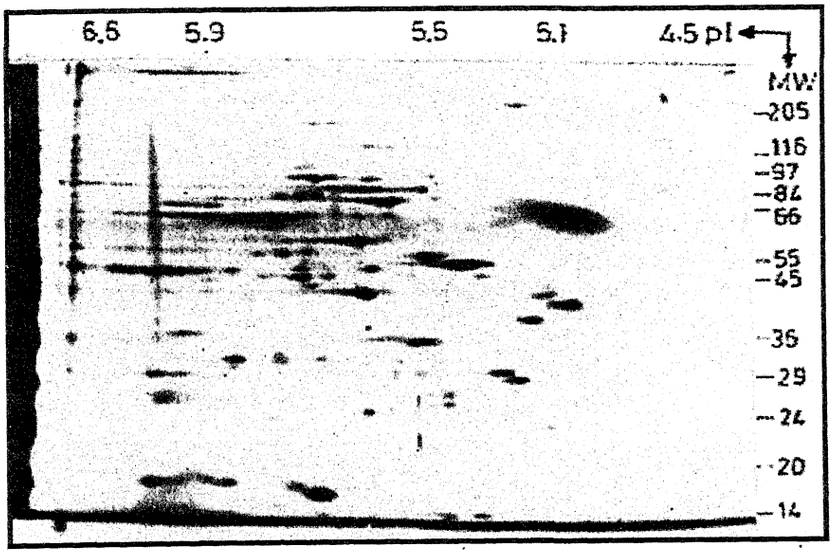
Accurate molecular masses, determined by MS have proved extremely useful in protein identification, but peptide mass fingerprinting is now the method of choice. On-gel digestion of proteins with endoproteinases followed by MS generates a highly specific peptide mass fingerprint. Comparison of the experimentally determined mass spectrometric peptide map with that theoretically calculated from the sequences in the databases leads to identification. The peptide map searches can be obtained by including partial sequence information derived from MS/MS of selected peaks. N-terminal sequencing by Edman-degradation, total amino acid composition and pI have also been used for protein identification and algorithms are now available that can combine the output from several different searches. If homology searches fail, PCR on cDNA libraries using degenerate primers derived from the partial sequences can be attempted. Other methods for protein characterization include single and double radiolabeling followed by phosphor imaging, or transfer to polyvinylidene difluoride (PVDF) membranes followed by immunoblotting or T-cell mitogen assays as well as monosaccharide or phosphate analysis[22].

Applications

Proteins determine the biological phenotype of an organism and are the primary targets for most therapeutic agents. Proteomics has the added advantage that it can be used to identify post-translational modifications of proteins (such as phosphorylation, glycosylation, acylation and methylation)[23]. Such modifications are vital for the correct functioning of many proteins and are being increasingly recognized as a major mechanism in cellular regulation. Proteome analysis captures all such changes and, therefore, provides unique and valuable information independent from, but complimentary to, genomic data[22].

Proteomics offers a major new approach to drug and vaccine discovery. The total number of proteins that can potentially be synthesized by an organism represents the conceptual proteome (≈100000 in a

a.



b.

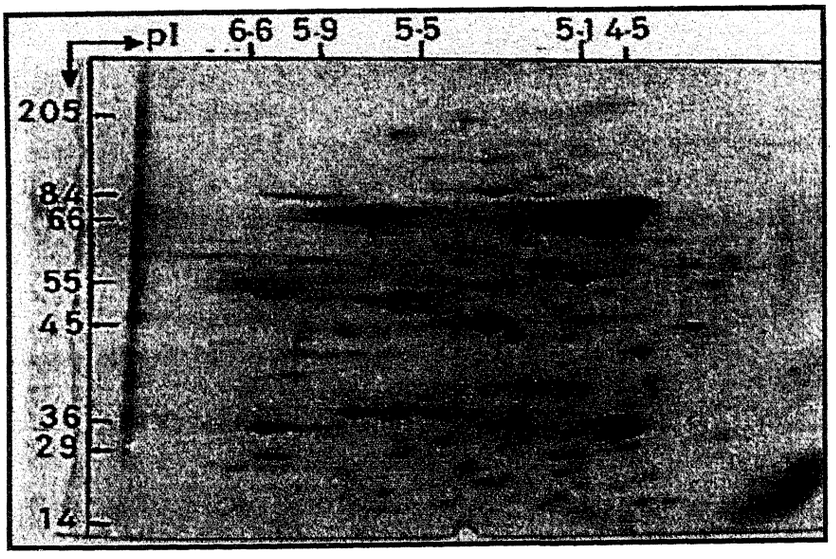


Fig. 3. 2-Dimensional protein maps of *Brugia malayi* (a) Adult soluble antigens and (b) Microfilarial antigens

"DNA makes RNA, RNA makes protein, and proteins make us."

Francis Crick

typical multicellular organism)[24]. At any one time, approximately 10 000 - 20 000 genes are being expressed selectively to yield the sets of proteins required for normal function (the experimental proteome). How these genes are regulated and how these regulation changes in response to drug treatment or other physiological or developmental changes constitute the dynamic output of the genome, and their study forms the next step in functional genomics[22].

Proteome analysis can be integrated with genome mapping projects and protein characterization studies by pointing to proteins that are novel, present in unusual amounts or have highly specific roles. It can assist in the confirmation of DNA open reading frames, in the characterization of knockout mutants and in the validation of drug and vaccine targets. Proteome maps are also useful reference points for future studies on temporal or spatial protein synthesis in identifying sex- or stage-specific proteins, excretory/secretory proteins and quantifying stress responses[22]

Human proteome organization & human proteome projects

26th June 2000 will be remembered as one of the most defining moments in the history of Science as this was the day that the formal announcement of the completion of the rough draft of human genome was made. The two draft sequences of the human genome were generated by the Human Genome Project (HGP)[25] and Celera Genomics[26]. With the human gene sequence in hand, researchers have now moved in to a new goal: identifying the proteins in the body[27]

HUPO (Human Proteome Organization), an international coordinating group has been launched in 2001. This is a loosely knit federation of proteome researchers. HUPO helps set priorities, coordinate research, set standards for handling and processing samples, and arrange for the use of common bioinformatics to ensure that researchers can directly compare their results. The organization has targeted blood plasma as its first priority, aiming to discover blood-borne proteins indicative of disease. The $1 million pilot project was launched in April 2002 and currently consists of researchers from 47 labs around the globe, including 28 in the United States[27].

Four separate public international proteomics initiatives have been launched over the past year and a half. Three of them spearheaded by researchers in the United States, Germany, and China, are aimed at tracking down all the proteins in human blood plasma, the brain, and liver. A fourth effort seeks to create antibodies against thousands of human proteins, a resource that should help researchers devise other protein-tracking tools. Additional proteome projects are looming, with kidney, muscle, heart, and saliva among the possible targets[27]

Major initiatives of HUPO

1. *PPP (Plasma Proteome Project)*

The PPP is focussed on comparing the strengths and weaknesses of different protein-hunting technologies, such as 2-D GE and liquid chromatography for separating proteins, and various versions of mass spectrometry for identifying them. HUPO leaders plan to set recommendations about which technologies are most

appropriate for tracking down different subsets of plasma proteins.

Knowledge about the housekeeping plasma proteins, such as albumin, fibrinogen, immunoglobulins and others that are present in large concentrations, is well established. However, the identification of peptides and proteins with regulatory function is difficult because they occur at low concentrations and within a complex mixture of the main plasma proteins[28]. Sometimes they are not completely solubilized to be seen on traditional 2-D maps [11]. Though the high-abundance proteins pose a major problem in staining gels for resolving other low molecular weight proteins, it has been found that they act like molecular sponges and readily bind a wide variety of low-abundance proteins (341 different proteins and peptides bound to albumin alone). Therefore, it might be possible to use common proteins to track down the low-abundance proteins and peptides that most groups are interested in as potential diagnostic markers [27].

Several other body fluids like urine, lachrymal fluid or saliva are also rich in peptides and are of interest for diagnostic purposes. For example, several biochemical markers in urine are used to measure bone turnover[29]. Application of surface-enhanced laser desorption–ionization (SELDI) technology could help in the identification of defensin peptide of 3.4 kDa in urine as a biomarker for transitional cell carcinoma of the bladder[28]. Specific peptides in saliva have been linked to diseases of teeth (e.g. Caries), and human breast milk is a rich source of many peptide growth hormones and antibiotics. Similarly, cerebrospinal or synovial fluid are some of the less accessible liquid subsystems in the body. CSF is rich in neuropeptides and is of relevance to neurodegenerative diseases[30]

2. *HBPP (Human Brain Proteome Project)*

HBPP begun as pilot project in April, 2003 and it builds on a brain proteome project that begun in 2000 and backed by $17 million from the German Government. It aims to sort out technology and standard issues, focussing at first on tracking down the proteins in the substantia nigra and hippocampus; the brain regions that degenerate in Parkinson's and Alzheimer's diseases. HBPP researchers hope to find proteins that mark the early disease stages, because most damage to brain cells occurs before the first symptoms show up. If these markers are found it can be studied to see if these proteins are visible in people of 30 or 40 years age or what is the age of their appearance or if there is a change in their abundance and how it correlates with the appearance of the early symptoms of the disease. The HBPP team also plan to analyze the cerebrospinal fluid (CSF) and blood plasma for proteins linked to brain diseases [27].

Early studies by Joachim Klose, a protein chemist at Humboldt University in Berlin and the HBPP co-director, in mouse model have reported that there is change in the abundance of the 250 brain proteins as the mice grew from embryos to aging adults. The overall amount of proteins remained essentially constant from a few days after birth until the animals died. Nearly 20% of the brain proteins continued to change their abundance levels when the animals were in the final stages of life. The results, Klose says, suggest that changes in members of this protein subset could be linked to disease.

3. *HLPP (Human Liver Proteome Project)*

HLPP is backed by an initial round of $25 million in funding from the Chinese government for a 3-year pilot study to be completed in 2005. The effort was launched in May and is aimed at setting up the collaborations, standards, and procedures for tallying the thousands of proteins expressed in human liver cells. So far, 79 labs, 37 of them in China, have signed on to the liver proteome effort. The HLPP aims to ultimately link liver-specific proteins to diseases such as hepatitis and liver cancer[27].

4. *Library Of Antibodies Against Human Proteins*

HUPO's fourth initiative is to make vast library of antibodies against human proteins. It is a massive task to raise antibodies against 10,000 human proteins; however, such antibodies could enable the development of high-speed protein-tracking technology, such as the antibody micro-arrays produced by 'Speed boost'. Such antibodies could be used to track a particular protein in many people as a way of confirming its involvement in a disease[27]

Future perspectives

Proteomics provides a powerful set of tools for the large-scale study of gene function directly at the protein level. In particular, the mass spectrometric study of gel-separated proteins is leading to a renaissance in biochemical approaches to protein function. Clinical and bedside applications of proteeomic research are an exciting component of the field of proteomics. New types of proteomic diagnostic technologies are being developed and heralded the beginning of what promises to be a revolution in molecular medicine[17]. Because proteins are one step closer to function than are genes, these studies lead directly to biological discoveries or hypotheses.

Human needs and their natural instinct to unravel the mystery have propelled most scientific researches. Louis Pasteur's pioneering research on anaerobic oxidation and alcoholic fermentation by the Brewer's yeast was a starting point in Biochemistry that was backed by the French wine industry with a motive to prevent their wine turning sour. Similarly, the HLPP initiative by the Chinese government stems from the fact that liver diseases kill hundreds of thousands of people in China every year. The HBPP is propelled by the neurodegenarative diseases like Parkinson's and Alzheimer's diseases that are a major problem in the Western world. However, who so ever takes the initiative, the beneficiary is the whole mankind.

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Admixture dependent doses prepared from the medicinal plants by "Kol" tribe for treatment of diabetes*

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Abstract

Different portions of the plants Jarul, Sadaphuli, Anjan and Nux-vomica (of the families Lythraceae, Apocynaceae, Simaroubaceae, Loganiaceae respectively) are reported as being used by "Kol" tribe for treating different diseases. This paper reports treatment practices adopted by Kols for treating diabetes. Survey reveals that the tribal customary wisdom, where drugs derived from different plants are used " in-combination"(i.e. Admixture Dependent Doses, ADD), is more effective.

(Keywords : diabetes/Kols/medicinal plants/ADD)

Plants have formed the basis of sophisticated traditional medicine system that has been in existence for thousands of years[1,2]. The first records, written on clay tablets in cuneiform, are from Mesopotamia of about 2600 B.C.; among the substances which they used were oils of *Cendrus* species (cedar), *Cupressus semipevirens* (cypress), *Glycyrrhiza glabra* (licorice), *Commiphora* species (myrrh) & *Papaver somniferum* (poppy juices), all of which are still in use today for the treatment of ailments ranging from cough & cold to parasitic infections & inflammation[3]. For many wild species of medicinal plants, suitable cultural practices are known. Medicinal plants are viewed as possible bridge between sustainable economic development, affordable health care and conservation of vital biodiversity[4]. It can be assessed and utilized only after determining the present state of local and regional flora and fauna.

The tribals obtain considerable part of their daily food requirement and cure for diseases from wild plants. Many plants are used by Kols as medicines against various ailments. In our earlier studies we reported the medicinal use of about 60 plants by the Kols[5, 6]. The "Kols" live in and around Allahabad, between the parallels of latitude 24°-47'and 25°-47' North and Longitude 81°-19' and 82°-21'East.They are found in

*Data also presented in the Scientific Session of 73rd Annual Session of The National Academy of Sciences, India held at Ahmedabad in 2003.

majority of the villages of Shankargarh (Development Block) situated 55Km. south of Allahabad. Kols are of average height and predominantly have long and narrow heads with a long or oval face and a moderated broad nose.

Observation : An extensive survey was made between Sep-Nov, 2003. The plants, reported in the paper, were collected on the basis of informations gathered from Kols and others of Shankargarh development block. There are given in Table 1. Herbarium was prepared and nomenclature/ classification was confirmed as per International Code of Nomenclature. It was found that the different parts of these plants are used for different medicinal purposes and several diseases are being cured by the consistent and wise use of these plant parts. It was also observed that the same plant is being used for many diseases but the preparation of medicine from the different plant parts have different methodologies which also gives an idea that the chemical constituents of the extracts from different plant parts are possibly different. The wide use of same plant parts for a number of diseases also gives a clue of the role of concentration of the extract made from the

Table 1– Plants used for curing diabetes and related symptoms (use for other diseases are also mentioned).

No	Common name	Scientific name	Family	Plant part	Used for	References
1	Jarul *	*Lagerstroemia speciosa (L) Pers.*	Lythraceae	Bark and leaf	Diabetes, Purgative	7,8,9,10,12, 13,14, 24
				Fruit	White ulcer on tongue	
				Root	Bleeding, fever	
2	Sadaphuli*	*Catharanthus roseus*	Apocynaceae	Leaves	Diabetes	2,9,11,17,18, 19,20,21,22, 23,25,26
				Whole plant	Blood pressure, childhood leukemia, testicular cancer	
3	Anjan*	*Ailanthus excelsa*	Simaroubaceae	Bark and leaves	Debility (from diabetes) cure, Tonic for debility after delivery	7,8,9,10
4	Nux vomica*	*Stychnos nux vomica*	Loganiaceae	Leaves	Tonic, stimulant	8,11,15,16,17
				Seed	Eczema & other skin diseases	

Coloured diagrams of above four plants are illustrated in Plate1 (Figs. 1-4).

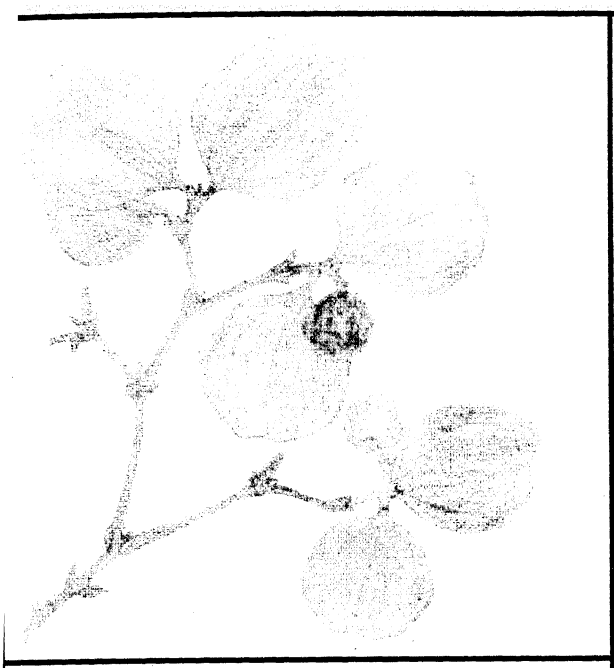

Fig. 1

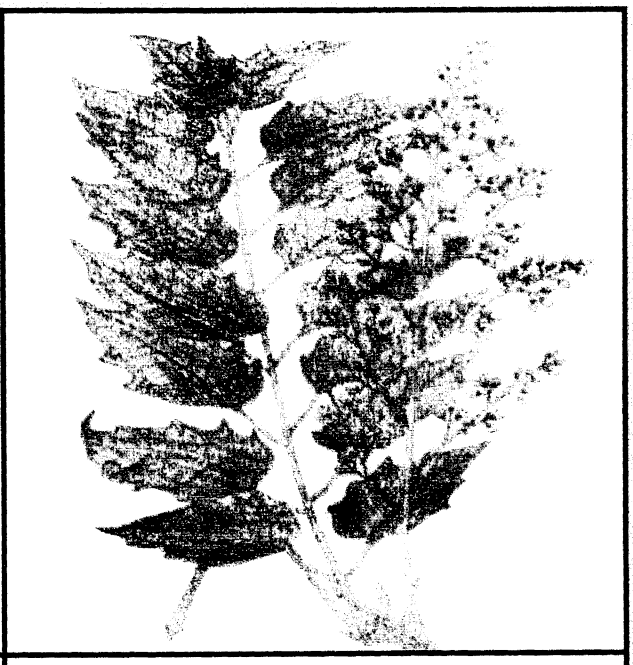

Fig. 2

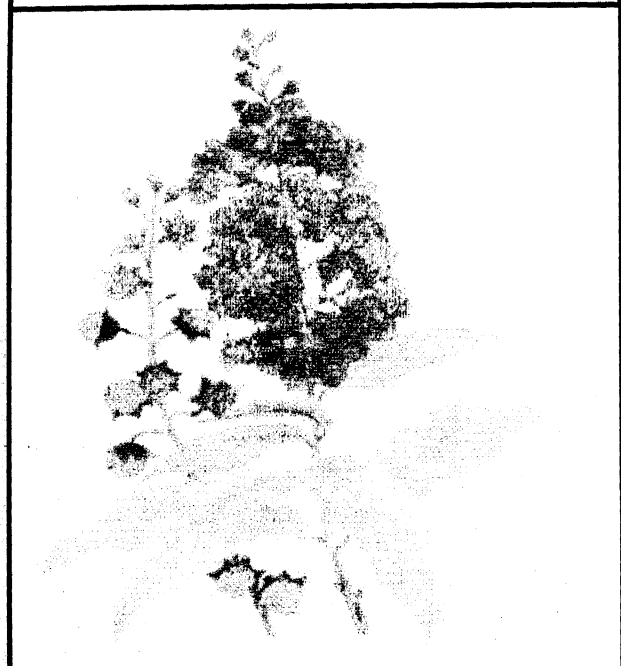

Fig. 3

Fig. 4

Fig. 1-4 - Coloured diagrams of 1. Nux-vomica, 2. The Tree of Heaven, 3. The Queen's Flower, 4. The common periwinkle

specific plant part, which is in coherence with the practice of allopathic medicines which are administered in different doses to different patients of different diseases along with the combination of other medicines. The preparation techniques also varies from people to people. We observed that the methods of preparing the extracts by the Kols with different intrinsic medicinal value generally vary.

Discussion : It was observed that Kols use Sadaphuli *(C. roseus)* and Jarul (*L. speciosa*) for curing diabetes. The leaves of both these plants are used for this purpose. Two other plants Anjan *(A.excelsa)* and Nux-vomica *(S.nux-vomica)* are also used in combination with Jarul and Sadaphuli for treating debility caused from diabetes. On the basis of their past experience, they prefer the use of Sadaphuli in the dawn. The idea of taking the fresh leaves (5-6 in number) of Sadaphuli at dawn is in coherence with the qualitative& quantitative preservation of its potency[27] at such low temperature and high humidity; as well as the availability of fresh air (O_2) at dawn and lower state of BMR of an individual supports better absorption of required molecule for enhanced immune response[28-29]. Jarul leaves/ bark are crushed to make paste and taken (20ml.) with milk in the night which not only acts as a medicine for diabetes but also a good purgative. The extract of Nux-vomica and Anjan leaves (10ml.approx.) are used for strengthening the system. Again Anjan is taken before every meal. Therefore it is evident that the Kols adopt multi pronged approach for treating diabetes and related weakness which includes different "timings" of body metabolic activity as well as admixture dependent doses (ADD). This type (multiple medicinal doses) of use is also prevalent in allopathic system of medicine where glycemic drugs (Chlorpropamide and Phenformin) are used in combination (as Chloroformin) along with other potentiating drugs (B-Complex and Vitamin A, E capsules etc.)[30].

Thus it could be suggested that ADD of medicinal plants yield better result for the cure of chronic diseases (as diabetes); and it should be adopted for preventive & promotive heath care.

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Modelling rice productivity : Additive nonparametric regression approach

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Abstract

Multiple linear regression (MLR) modelling is a very powerful technique and is extensively used in agricultural research. This methodology assumes a linear relationship between response and explanatory variables, which may not hold in reality. Recently, a very promising nonparametric regression methodology is emerging, which is of great help in situations where functional parametric relationship is not known. In this paper, this methodology for two explanatory variables is thoroughly discussed. Subsequently, as an illustration, the methodology is applied to model rice productivity of Faizabad district using fertilizer consumption and area under irrigation as explanatory variables. A comparison with conventional MLR approach shows superiority of proposed methodology for data under consideration.

(Keywords: rice productivity/ nonparametric regression/ additive model/ local linear regression smoother/ cross- validation)

In an excellent review, Khush and Baenziger[7] pointed out that globally 60% more rice and wheat will be needed by the year 2025 to feed additional population and to meet income-generated demand. Thus, technologies to increase productivity of both these crops are urgently needed. The additional food must be produced from existing land. Otherwise, there will be pressure to open up ecologically more fragile lands. As far as our country is concerned, rice is the most important staple food. It is grown under varied environmental conditions, presently covering 43.92 million hectares with productivity of 2066 kgs / ha (DES[2]). Increase in rice productivity would be mainly attained by adopting improved varieties and investment in existing irrigated area. The potential yield of high yielding varieties (HYVs) can be realized only under optimum soil conditions, viz. nutrient and water availability. A study conducted by F.A.O., Italy shows that there is a highly significant relationship between fertilizer consumption and crop yield. Irrigation is another crucial input in agriculture. Its use increases crop production as well as determines the nature of response for other inputs. Misuse of water not only leads to its wastage, but also reduces efficiency of applied fertilizers and other inputs (Chandra *et al.*[1])

Multiple linear regression (MLR) approach is the most extensively used statistical technique for modelling those situations where more than one factor influences response variable. The underlying model can be expressed as:

$$Y=a_0+a_1X+a_2Z+\varepsilon \quad (1)$$

where Y is response variable; X, Z are explanatory variables; and ε is the error term. The parameters a_0, a_1, and a_2 are estimated using "Method of least squares". A good description of various aspects of this methodology is given in Draper and Smith[3]. Lalitha *et al.*[8] used this technique to model tiller production in low land rice varieties using temperature and sunshine hours. Sisodia and Singh[11] employed MLR methodology for modelling rice production of Faizabad district. However, one drawback of this methodology is that linear relationship is assumed between response variable and explanatory variables, which may not hold in reality. In such a situation, an emerging powerful technique of nonparametric regression, which does not assume any parametric form for functional relationships between the variables, may be employed. In this paper, proposed methodology is thoroughly discussed for the case of two explanatory variables. Subsequently, it is applied to model rice yield of Faizabad district of U.P., India and its superiority over traditional MLR approach is demonstrated.

Methodology

Nonparametric regression methods help to analyze data without having to postulate a shape for relationship between response variable and explanatory variables (Efromovich[4]). Such methods are generally based on the assumption of additivity and are popularized by Hastie and Tibshirani[6]. Here, back-fitting algorithm is used to estimate underlying functions. Starting from an initial estimate for first explanatory variable, smooth function for the second is estimated. Then, underlying function corresponding to each explanatory variable is estimated iteratively until convergence is achieved. As convergence of back-fitting algorithm is not always assured, Opsomer and Ruppert[9] proposed a method for fitting bivariate additive model using local polynomial regression, which is discussed below in detail.

Consider an additive nonparametric regression model with explanatory variables X and Z:

$$Y_i=\alpha+m_1(X_i)+m_2(Z_i)+\varepsilon_i \quad (2)$$

where α is intercept and ε_i are independent and identically distributed with mean zero and variance σ^2. Assume $E\ [\ m_1(X_i)] = E\ [\ m_2(Z_i)] = 0$, where symbol E denotes the expected value. Estimator of parameter α is obtained as $\bar{Y}$. Now the analysis involves computation of estimators of $m_1(.)$ and $m_2(.)$. Let $m_1 = (\ m_1(X_1), \ldots, m_1(X_n))'$ and $m_2 = (m_2(Z_1), \ldots, m_2(Z_n))'$ and $m = m_1 + m_2$ and "Equivalent kernel" be defined as

$$S'_{1,x} = e'\,(X'_x W_x X_x)^{-1}\, X'_x W_x \quad (3)$$

where e' is a vector with two elements 0 and 1 and X_x is a $(n \times 2)$ - matrix with unity as the element in first column and $(X_i - x)$ as the i^{th} element of second column. The matrix W_x is a diagonal matrix with i^{th} element $[K(\ (X_i - x\ )/h\ )]\ /\ h$. Here h is bandwidth which decides degree of smoothing and $K(.)$ is kernel density function. Most commonly used kernel is Epanechnikov kernel given as

$$K_h\ (u) = \begin{cases} 0.75(1-u^2), & \text{for } |u| \le 1; \\ 0 & \text{otherwise} \end{cases}$$

Choice of optimum bandwidth is of great importance in nonparametric regression estimation. Cross - validation or leave-one-out method is the most commonly used technique. As proposed by Fan[5], estimate of Y_j corresponding to X_j is obtained by employing local linear smoother, without including j^{th} observation of Y. Thus, for each value of h, corresponding Y estimates and Mean Square Error (MSE) are computed. Optimum bandwidth is selected as the one for which MSE is minimum.

Similar to eq. (3), $S'_{2,z}$ can be expressed as

$$S'_{2,z} = e' (Z'_z W_z Z_z)^{-1} Z'_z W_z \qquad (4)$$

Let S_1 denote the matrix with S'_{1,x_i} in the i^{th} row and S_2 the matrix with S'_{2,z_i} in the i^{th} row. Now, $\hat{m}_1$ and $\hat{m}_2$ are computed as the solutions of estimating equations :

$$\begin{bmatrix} D & S_1^* \\ S_2^* & D \end{bmatrix} \begin{bmatrix} \hat{m}_1 \\ \hat{m}_2 \end{bmatrix} = \begin{bmatrix} S_1^* \\ S_2^* \end{bmatrix} Y \qquad (5)$$

where $S_i^* = (D - J/n) S_i$, D denotes identity matrix, and J is a $(n \times n)$-matrix with unity as all elements. On solving eqs. (5) :

$$\hat{m}_1 = \{D - (D - S_1^* S_2^*)^{-1} (D - S_1^*)\} Y$$

and

$$\hat{m}_2 = \{D - (D - S_2^* S_1^*)^{-1} (D - S_2^*)\} Y$$

Thus, the expression for $\hat{m}$ is obtained as

$$\hat{m} = \{J/n - 2D - (D - S_1^* S_2^*)^{-1} (D - S_1^*) - (D - S_2^* S_1^*)^{-1} (D - S_2^*)\} Y$$

Finally, from eq. (2), estimator of Y at each time epoch is computed as the sum of $\hat{m}$ and $\bar{Y}$.

As no software package is available for carrying out above computations, relevant computer programs are developed in MATLAB, Ver. 5.3.1 software package and are appended in Annexure - I.

Results and Discussion

As an illustration of the above methodology, data set as given in Singh[10] is considered. It comprises rice productivity data of Faizabad district of U.P., India from 1968 to 1995 as response variable and fertilizer consumption (X) and total irrigated area under rice crop (Z) as explanatory variables. The data is reproduced in first four columns of Table 1 for ready reference. In the first instance, Multiple linear regression (MLR) model, given in eq. (1), is fitted to above data using SPSS, Ver. 10.0 software package and the following results are obtained:

$$\hat{a}_0 = 551.14, \quad \hat{a}_1 = 9.11, \quad \hat{a}_2 = -0.04$$
$$(142.81) \qquad (2.46) \qquad (2.49)$$

where figures in brackets ( ) indicate corresponding standard errors. It may be noted that standard error of estimate of parameter a_2 is relatively very high. Mean Square Error (MSE) for fitted MLR model is computed as 72241. Estimated rice yield based on this model at each time epoch is reported in fifth column of Table 1.

Table 1– Estimated rice yield in Faizabad district, U.P. along with data.

Year	Rice Yield (kgs / ha)	Fertilizer Consumption (kgs / ha)	Irrigated area ('000 ha)	Estimated Rice Yield (kgs/ha)	
				MLR	ANR
1968	705	48.89	2.54	996	776
1969	733	64.00	1.94	1134	804
1970	618	41.42	4.49	928	802
1971	932	42.89	4.39	942	800
1972	921	39.21	4.62	908	804
1973	752	25.58	5.44	784	840
1974	735	32.16	5.18	844	822
1975	933	6.97	3.51	614	896
1976	919	48.57	4.02	993	804
1977	849	65.59	6.69	1148	906
1978	1130	83.15	11.26	1308	1046
1980	912	69.79	16.75	1186	1134
1981	1414	72.19	10.09	1208	995
1982	1268	92.62	26.05	1394	1413
1983	1379	104.55	27.75	1502	1492
1984	1507	78.67	29.13	1267	1446
1985	1765	93.75	30.72	1404	1528
1986	1404	79.47	34.76	1274	1588
1987	1526	89.83	34.76	1368	1617
1988	1788	116.24	35.13	1609	1705
1989	2099	124.16	45.52	1680	2051
1990	2113	109.30	48.40	1545	2105
1991	1958	143.82	93.29	1857	1947
1992	1806	116.47	114.57	1607	1819
1993	2132	211.64	139.92	2473	2128
1994	2153	182.57	160.13	2208	2098
1995	1836	171.23	150.91	2105	1920

Subsequently, additive non-parametric regression (ANR) model is fitted to given data. First step involves estimation of optimum bandwidth and is computed by cross-validation method using computer programs developed in MATLAB, Ver. 5.3.1 software package. These programs are available with the authors but are not included here to save space. For present data, optimum bandwidth is estimated as 0.19. Further, using computer programs given in Annexure - I, estimates of Y at each time-epoch is computed and are given in last column of Table 1. Corresponding MSE is computed as 19061, which is found to be much lower than that for MLR model indicating superiority of the proposed methodology. Finally for visual display, graph of fitted model by ANR approach along with data, is exhibited in Fig. 1.

Conclusions

In this paper, efforts are made to apply additive nonparametric regression approach to model a data set when two explanatory variables are present. This methodology is shown to be extremely useful when a specified functional form for underlying model is not known. As there are hardly any underlying assumptions, this methodology is flexible and robust as compared to parametric approaches, like multiple linear regression. It is hoped that scientists would start applying "Additive nonparametric regression approach" to investigate underlying relationships in their data sets. Work is in progress to extend this approach when there are more than two explanatory variables and shall be reported separately in due course of time.

Annexure- I

Program for additive nonparametric regression methodology

```
fd=fopen('c:\data\additive.xls','w');
fd1=fopen('c:\data\additive27.txt','r');
n=27;
[A,count]=fscanf(fd1,' %f %f %f \n');
```

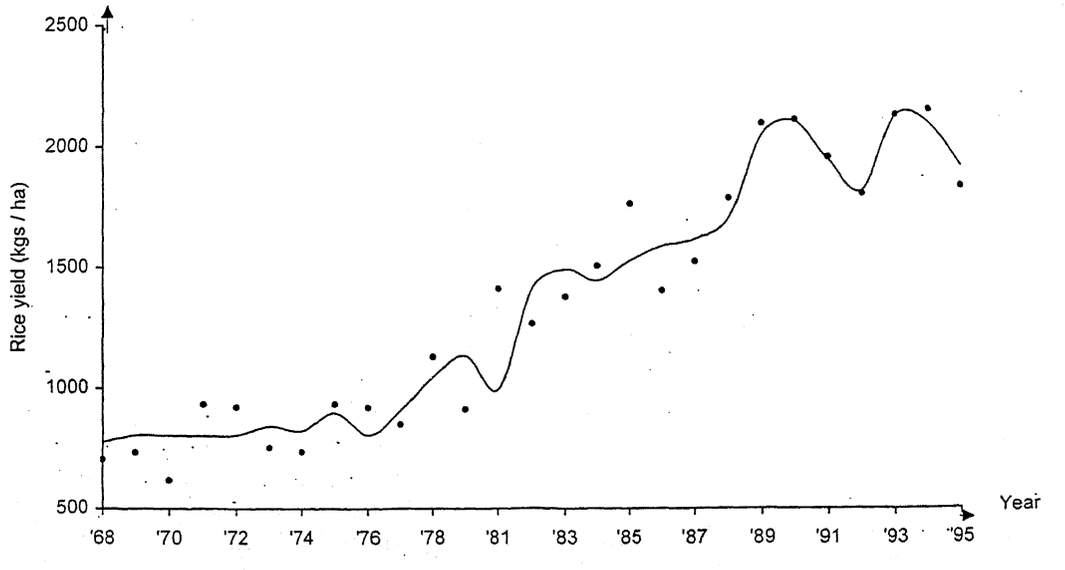

Fig. 1–Fitted additive nonparametric regression model for rice yield of Faridabad district along with data

```
for i=1:n
z1(i)=A(i*3-1); z2(i)=A(i*3); y(i)=A(i*3-2);
end;
mean=sum(y)/n;
for i=1:n
x(i)=(z1(i)-min(z1))/(max(z1)-min(z1));
v(i)=(z2(i)-min(z2))/(max(z2)-min(z2));
end;
for i=1:n
   h=0.19;                a0=0; a1=0; a2=0;
 for j=1:n
    u1=(x(j)-x(i));   u=u1/h;
kr(j)=0.75*(1-u*u);
     if abs(u)>1
       kr(j)=0;
     end;
   a0=a0+kr(j);    a1=a1+u1*kr(j);
a2=a2+u1*u1*kr(j);
 end;
 for k=1:n
  w(i,k)=kr(k)*(a2-(x(k)-x(l))*a1)/(a0*a2-
a1*a1);
 end;
end;
s1=w;
for i=1:n
 h=0.19;    a0=0; a1=0; a2=0;
 for j=1:n
  p1=(v(j)-v(i));      p=p1/h;
kr(j)=0.75*(1-p*p);
  if abs(p)>1
   kr(j)=0;
  end;
  a0=a0+kr(j);        a1=a1+p1*kr(j);
a2=a2+p1*p1*kr(j);
 end;
 for k=1:n
   w(l,k)=kr(k)*(a2-(v(k)-v(l))*a1)/(a0*a2-
a1*a1);
 end;
end;
s2=w;
for i=1:n
 for j=1:n
   o1(i,j)=0;        o2(i,j)=1/n;
   if (i==j)
     o1(i,j)=1;
   end;
 end;
end;
s1=(o1-o2)*s1;   s2=(o1-o2)*s2;
m1=(o1-inv(o1-s1*s2)*(o1-s1))*y';
m2=(o1-inv(o1-s2*s1)*(o1-s2))*y';
m=m1+m2;
for i=1:n
 fprintf(fd,'%d %10.4f %10.4f %10.4f
%10.4f\n',i,m1(i),m2(i),m(i)+mean,y(i));
end;
st=fclose(fd);
```

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Genus *Arachniopsis* Spruce—new to India with *A. indica* sp. nov. as new to science

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Abstract

The genus *Arachniopsis* Spruce (subfamily Zoopsidoideae) is being reported for the first time from India (Governor Sholai, Nilgiri hills, Tamil Nadu). The Indian plants appear to be different from the known species of the genus hence are being described as *A. indica* sp. nov.

(Keywords : new species / Zoopsidoideae/ *Arachniopsis indica*)

The Nilgiri hills in peninsular India represent a rich diversity area of bryophytes both in frequency and variety. Some of the highly nourished localities and sites like Governor Sholai (Ootacamund) come under Mukurthy National Park (a reserve forest) of Nilgiri District of Tamil Nadu. The entire area is covered by sub-tropical evergreen forests, which receive adequate rainfall with luxuriance of terrestrial and epiphytic bryophytes. In a recent exploration of the area in the month of April 2002, some plants answering to genus *Arachniopsis* Spruce, growing on slightly acidic soil have been discovered for the first time from India. The plants require cool, shaded and almost continuously moist habitat for their growth and development. The genus is not very frequent and escapes the attention due to very small size generally invisible by naked eye. Detailed microscopic studies revealed the first ever record of this genus from India.

The genus *Arachniopsis*, introduced by Spruce in 1882, comes under most primitive subfamily Zoopsidoideae of family Lepidoziaceae which consists of some very small and simplified genera like *Zoopsis* Hook., *Pteropsiella* Spruce, *Bonneria* Fulford and Tayl., *Pseudocephalozia* Schust. and *Telaranea* Spruce. *Arachniopsis* is mostly distributed in Gondwanaland. Most of the species are distributed in Neotropical, Oceania and Antilleas region of the world. *A. diplopoda* Pócs and *A. diacantha* (Mont.) Howe are distributed in Neotropics, east African Islands and sub-Saharan Africa[1,2,3,4,5,6]. *Arachniopsis sejuncta* (Ångstr.) Schust., *A. dissotricha* Spruce, *A. tenuifolia* Schust., *A. caduciloba* Schust., *A. confervifolia* (G.) Howe, *A. pecten* var. *confervoides* Schust., *A. pecten* var. *pecten* Spruce, and *A. anomala* Schust. are strictly Neotropical in distribution[10], while *A. monocera* Schust. *et* Grolle is distributed in Oriental region, *A. major* Herz. in Oceania and *A. borinquena* Schust. is the only species which is Antilleas in distribution [10].

Contribution from the Department of Botany, University of Lucknow, Lucknow (Bryophyta) no. 265.

The genus *Arachniopsis* Spruce has been treated under two subgenera, *Arachniopsis* subgen. *Arachniopsis* and *Arachniopsis* subgen. *Amphidactylopsis* Schust. Subgenus *Monodactylopsis* Schust.[11] is now regarded as an autonomous genus[10].

The genus comprises of some most specific characters of simplification or progressive reduction in attaining delicate stem and elongated leaves which consist of 2 – 3 lobes and the axial anatomy consisting of one central and 5 peripheral cells is highly reduced among Jungermannialean taxa. Owing to these characters the genus is placed under the subfamily Zoopsidoideae of Lepidoziaceae which is represented in India by two genera *Lepidozia* (Dum.) Dum. and *Kurzia* v. Mart.[12].

Arachniopsis indica is placed under section *Arachniopsis* of subgen. *Arachniopsis* due to presence of caducous, transversely arranged 1–2 lobed leaves, vertical lobe with flaccid cells and axis with 1-3 medullary cells.

The present communication adds one more genus under the family Lepidoziaceae and provides an illustrated account of genus *Arachniopsis*, a new record for India as well as *A. indica* sp. nov. as new to science.

Arachniopsis indica sp. nov. (Plate: 1, Figs.: 1 - 12; Plate: 2, Figs.: 1 - 6)

Planta minor, flaccida, exsiccata collabens, cellulae corticalis 5, cellula medullosus 1; Folia caulina bi-tri loba, lobus uniseriatus, 2-5 cellulae longae, insertio lateralis et transversalis, discus basilis absens. Amphigastria linearis vel bilobus. Reproductio asexualis ab gemma.

Plants delicate, light green (whitish), glistening, erect, pellucid (plants collapse when dry), transparent, up to 3.0 mm long and 0.09 mm in width. Stem up to 40 μm across, differentiated in 5 cortical and 1 medullary cells, dorsal cortical cells large, 2 in number, 15 x 12 μm; ventral cortical cells small, 3 in number, 12 x 10 μm; medullary cell one, 10 x 7 μm; branching ventral intercalary. Leaves distant, succubous, transversely inserted, bilobed (-trilobed), leaf-lobe uniseriate, free (no disc formation), 2-3 cells long, rectangular: leaf-lobe cells thin-walled, 0.05 – 0.07 x 0.020 – 0.022 μm. Gemmae bicelled, at terminal end of leaf lobes near shoot apex. Underleaves smaller than leaves, vestigial, 1-2 lobed, 1-3 cells in length. Asexual reproduction by two celled gemmae, 14.5 – 15 x 10 – 12.5 μm.

Typus: HOLOTYPE, South India: Tamil Nadu – Nilgiri hills (Ootacamund – Governor Sholai); alt. ca. 2250 m.; 10.04.2002; P.K. Verma, A. Alam and N. Sahu; 15496/2002 (LWU).

Range: Endemic to India (restricted only in Nilgiri hills).

Ecology: The plants are hygrophilous in nature, require cool, moist and shady habitat, growing on acidic substrate under tree canopy, intermixed with *Jungermannia appressifolia*, *Plagiochila indica* and Mosses.

Phytogeographically this taxon occurs in the area of Deccan plateau in one of the oldest hill ranges of Gondwanaland in Peninsular India which is also a center of many endemic species. *Arachniopsis* is a significant addition to this part of the world. The species is an example of progressive simplification or reduction in number of cells in axial anatomy (i.e., 5 cortical and 1 medullary cell) and leaf-lobe

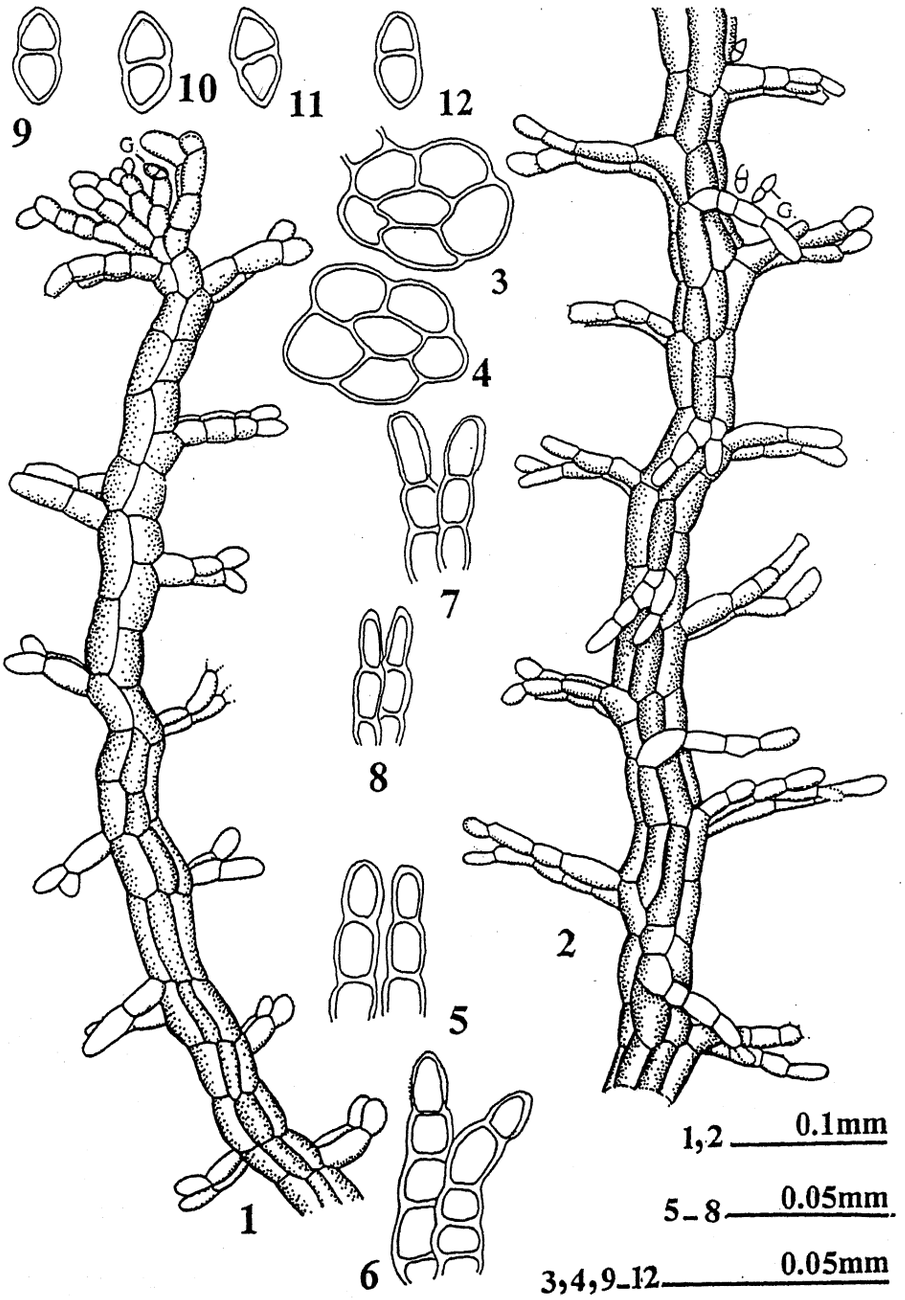

Plate 1– *Arachniopsis indica* sp. nov., figs.: 1 – 12. 1. Plant (dorsal view); 2. Plant (ventral view); 3,4. Stem, T.S.; 5-8. Leaves (leaf-lobe); 9-12. Gemmae.

because acroscopic end of merophyte is not utilized in leaf formation thus resulting into a leaf-free from base (i.e., no basal disk is formed). Most of Jungermannialean taxa have basal disc. Simplification also involves malleability in leaf-lobe (2 to 3) number[10,13,14,15].

Arachniopsis indica resembles *A. borinquena* Schust. of same section (*Arachniopsis*), in having 5 + 1 axial anatomy, collapsing nature of leaf-lobes and transversely inserted leaf but differs in having asexual devices that is bicelled gemmae (absent in *A. borinquena*) and shorter length of leaf lobe segment (2-3 cells long in *A. indica* while 6 –8 cells long in *A. borinquena*).

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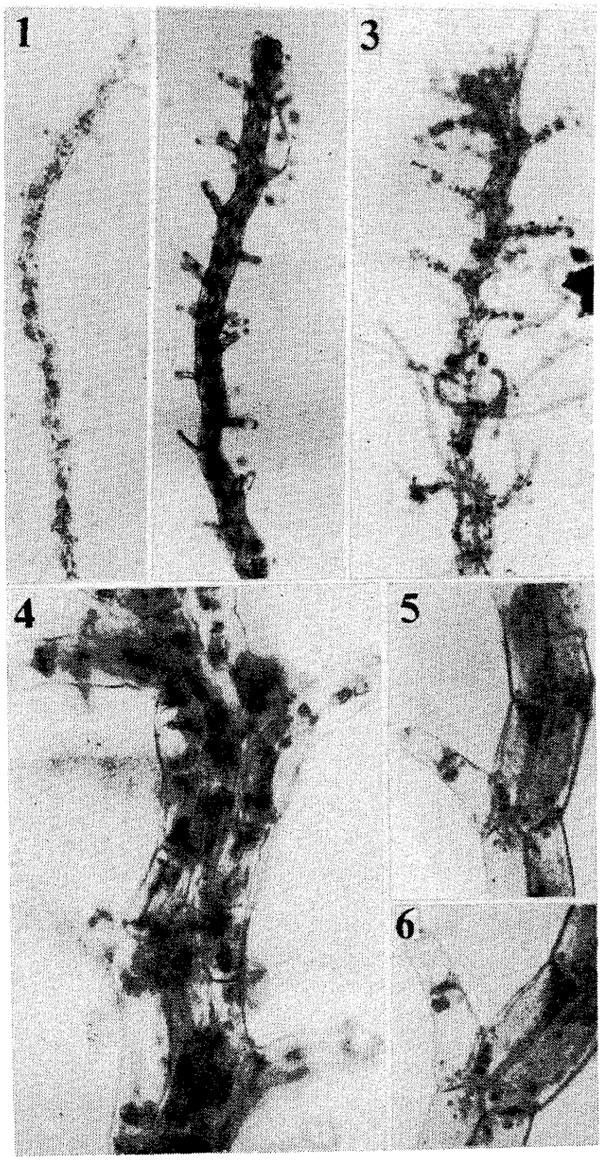

Plate 2– *Arachniopsis indica* sp. nov., figs.: 1 – 6. 1,2. Plants, dorsal view (x250); 3. Plant, ventral view (x250); 4. Plant portion, enlarged (x600); 5. Lateral leaf lobe (x600).

Light induced transformation of quinine : Studies in the effect of pH

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Abstract

Benzophenone sensitized irradiation of quinine (I) by UV light produces (II) by dimerization in neutral medium and at pH 4.0 gives products (III), (IV) and (V). In alkaline medium it is resistant to UV light. The structures of the products have been confirmed by spectral and elemental analyses.

(Keywords : UV/phototransformation/quinine)

Introduction

Photochemistry of organic compounds has been a vast field of research since a long time. Photochemistry of alkaloids has also been studied by some workers[1-10]. But the photolysis of quinine has not been studied so far. Therefore, here we report the photolysis of this alkaloid. Quinine being medicinally important compound, its photochemical studies are of significance.

Materials and Methods

Quinine (2.0 g) was dissolved in dried and distilled ethanol (200 ml). Benzophenone was added as sensitizer and the solution was irradiated by low pressure mercury vapour lamp (125 , W). The progress of the reaction was monitored by TLC in ethanol. After about 39 h a new spot appeared on the plate and that of the original became very faint. Then the irradiation was stopped. The product II was separated by preparative TLC and was recrystallized from ethanol (Yield : 1.0g; m.p. 230°C; Found C 76.40, H 7.38, N 4.66; Calculated for $C_{36}H_{40}N_2O_4$, C 76.59, H 7.07, N 4.96%).

Irradiation in acidic medium :

The reaction was carried out in same manner as above, but the solution was made acidic by adding dil. HCl and the pH was recorded to be 4.0. The reaction completed in 50 h. The reaction mixture was neutralized and concentrated by distillation on water bath under reduced pressure. The product were separated by preparative TLC (Yield : 0.8g; m.p. 168-170^{0}C; Found C 70.01, H 5.30, N 7.40; Calculated for $C_{11}H_{11}NO_2$, C 69.84, H 5.82, N 7.4%.

The reactions were carried out at different pH, but no change in the nature of products was observed.

Irradiation in alkaline medium :

No change was observed on TLC plate even after 50 h of irradiation.

The IR spectra were recorded in KBr pallets and NMR in $CDCl_3$ at 300 MHz.

Results and Discussion

Quinine (I) when irradiated by UV light at neutral pH, gave (II) (Scheme I) by dimerization. Its I.R. spectrum shows important absorption bands at 3250 cm^{-1} (OH stretching); 3030 cm^{-1} (C-H stretching arom.); 2926 cm^{-1} (C-H stretching), 1600 cm^{-1} (C=N stretching); 1425-1450 cm^{-1} (C-H bending); 1400-1550 cm^{-1} (C=C stretching benzene ring) etc. The H NMR spectrum gives signals at δ 1.6-3.3 (alicyclic protons), δ 3.7 (CHOH protons), δ 4.1 (OCH_3 protons), δ 4.2 (OH protons) and δ 7.5-9.0 (arom protons). The ^{13}C NMR spectrum gives signals at 19.2-60.9 (alicyclic carbon atoms), 68 (C-OH carbon) 70 (O-CH_3 carbon) and 121-149.6 (arom carbon atoms). The mass spectrum gives molecular ion peak at *m/z* 564.

Quinine (I) when irradiated by UV light at pH 4.0 gave products (III), (IV) and (V). The identity of pyridine (IV) has been established by co-tlc with the authentic sample. The structure of (III) has been confirmed by spectral and elemental analysis. The results of elemental analyses are given in experimental section and are in accordance with the calculated value. The IR spectrum of III shows important absorptions at 3228 cm^{-1} (OH stretching), 2935 cm^{-1} (C-H stretching), 1612, 1506 cm^{-1} (arom. multiple bond stretching), 1110 cm^{-1} (C-O-C stretching) etc. The ^1H NMR gives signals at 2.5 (-CH_2 protons), δ 4.0 (OH proton), 3.4 (OCH_3 protons) and 7-8 (arom. protons). The ^{13}C NMR spectrum shows signals at 58.5 (CH_2OH carbon atom), 120-7-150 (arom. carbon atoms) and 65 (OCH_3 carbon atom). The mass spectrum gives molecular ion peak at *m/z* 189.

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On the degree splitting graph of a graph

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Abstract

In this communication we define degree splitting graph of a graph and we study some properties of degree splitting graph.

(Keywords : graph / complete graph / complete bipartite graph/ regular graph)

Introduction

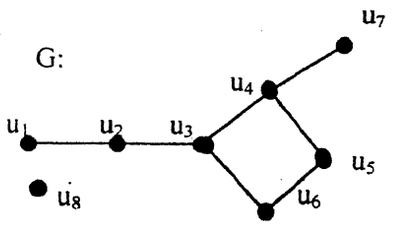
By a graph $G=(V,E)$ we mean a finite undirected graph. A graph G is said to be r regular if all the vertices of G have the same degree r. A maximal connected sub graph of G is called a component of G. For a vertex v in $V(G)$ we denote the degree of v by $d(v)$. K_n denotes the complete graph on n vertices. The addition of two graphs G_1 and G_2 is a graph G_1+G_2 with $V(G_1+G_2) = V(G_1)\cup V(G_2)$ and $E(G_1+G_2) = E(G_1)\cup E(G_2) \cup \{uv : u\in V(G_1), v\in V(G_2)\}$.

Splitting graph was introduced by Sampathkumar and Walikar[1]. For each vertex v of a graph G, take a new vertex v' and join v' to all vertices of G adjacent to v. The graph $S(G)$ thus obtained is called the splitting graph of G. On similar line, we define degree splitting graph $DS(G)$ of a graph G and we investigate some properties of $DS(G)$. Terms not defined here are used in the sense of Harary[2], Bondy and Murty[3]

Degree Splitting Graph

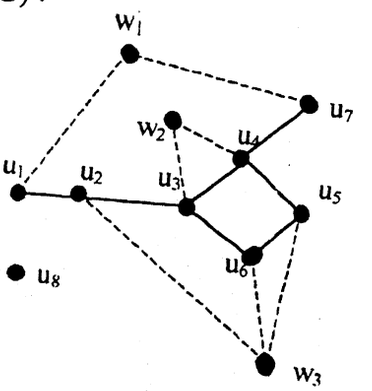
Definition 1 : Let $G=(V,E)$ be a graph with $V=S_1\cup S_2 \cup \ldots\ldots \cup S_t \cup T$ where each S_i is a set of vertices having at least two vertices and having the same degree and $T = V \setminus \cup S_i$. The degree splitting graph of G denoted by $DS(G)$ is obtained from G by adding vertices $w_1, w_2, \ldots\ldots, w_t$ and joining w_i to each vertex of S_i $(1 \le i \le t)$.

Example :



Here $S_1=\{u_1,u_7\}$, $S_2=\{u_3,u_4\}$, $S_3=\{u_2, u_5, u_6\}$, $T=\{u_8\}$.

DS(G) :



Note 1 : For any graph G, G is a subgraph of $DS(G)$. Clearly if G contains atleast two vertices, then G contains atleast two vertices of the same degree. Hence $G=K_1$ is the only graph such that $G=DS(G)$.

2. If G is regular, then $DS(G) = G+K_1$.

Notation : $d^*(v)$ denotes the degree of a vertex v in $DS(G)$. Clearly $d(v) \leq d^*(v)$ for all v in $V(G)$.

Theorem 1 : Let G be a graph with p vertices and q edges and let r be the number of vertices in T, where T is as in definition 1.

Then $|E(DS(G))|=p+q-r$.

Proof : Let $V(G) = \{u_1,u_2,.....u_p\}$ and $V(DS(G))\backslash V(G)=\{w_1,w_2,....,w_t\}$.

$$\text{Now } \sum_{i=1}^{P} d^*(u_i) = \sum_{i=1}^{P} (d(u_i)+1) - r \qquad (1)$$

$$\text{and } \sum d(w_i) = p-r \qquad (2)$$

From (1) and (2)

$$\sum_{v \in DS(G)} d^*(v) = \sum_{i=1}^{P} (d(u_i)+1) - r + p - r$$

$$= \sum_{i=1}^{P} d(u_i) + P - r + p - r$$

$$= 2q + 2(p-r)$$

Therefore, $|E(DS(G))| = p+q-r$

Corollary 2 : If G is k – regular, then $|E(DS(G))| = p(k+2)/2$.

Proof : Put $r = 0$ and $q=pk/2$ in the above theorem.

Note that if $G = K_n^c$, then $DS(G) = K_{1,n}$. Next we prove that $G = K_n^c$ is the only graph such that $DS(G)$ is a cyclic.

Theorem 3 : Let G be a graph with $|E(G)| \neq 0$. Then $DS(G)$ contains a cycle.

Proof: *Case (i)* : Let G be a tree.

Let u and v be the vertices of G such that $d(u)=d(v)=1$. Since G is connected, G contains a unique u-v path say $u=u_1\ u_2$ ....., $u_n = v$, Now $d(v) = d(u)$. By the definition of $DS(G)$, $DS(G)$ contains a vertex w such that w is adjacent to both u and v. Hence $wu_1\ u_2$ ..... u_nw is a cycle in $DS(G)$.

Case (ii) : Suppose G is not a tree.

If G contains a cycle, then $DS(G)$ also contains that cycle.

Suppose G is acyclic, then any component H of G is a tree. By case (i), $DS(H)$ contains a cycle. Hence $DS(G)$ contains a cycle.

Theorem 4 : Let G be a bipartite graph with bipartition $X = \{u_1,u_2, ..,u_m\}$ and $Y = \{v_1, v_2,.....,v_n\}$.

(i) If there is a pair u_i and v_j such that the length of the $u_i - v_j$ path is odd and $d(u_i) = d(v_j)$, then $DS(G)$ is not bipartite.

(ii) If there is no pair u_i and v_j such that $d(u_i) = d(v_j)$, then $DS(G)$ is bipartite.

Proof : (i) Suppose there exists vertices $u_i \in X$ and $v_j \in Y$ such that $d(u_i) = d(v_j)$ and the length of u_i-v_j path is odd. In $DS(G)$, there exist a vertix w such that w is adjacent to both u_i and v_j. Hence $DS(G)$ contains a cycle of odd lenth. Therefore, $DS(G)$ is not bipartite.

(ii) Suppose there is no pair u_i and v_j such that $d(u_i) = dv_j)$.

Let$V(DS(G))\backslash V(G) = \{w_1,w_2,.....w_t\}$. By the given hypothesis, no vertex in the above set is adjacent to both the vertices u_i and v_j. Without loss of generality, we can assume that $V_1 = \{w_1, w_2,.....,w_r\}$ is a set of vertices adjacent to vertices in $\underline{Y}$ and V_2 $\{w_{r+1}, w_{r+2}, ..., w_t\}$ is a set of vertices adjacent to vertices in X. Clearly $(X\cup V_1, Y\cup V_2)$ is a bipartition of $DS(G)$.

Corollary 5 : $DS(K_{m,n})$ is bipartite if and only if $m \neq n$.

Theorem 6 : $\omega\ (DS(G)) \leq \omega(G)$, where $\omega(G)$ denotes the number of components of G.

Proof : Let $V(G) = \{u_1,u_2,.....u_p\}$ and $V(DS(G)) \setminus V(G)=\{w_1,w_2,..w_t\}$.

Case (i) : Let G be connected.

Consider the arbitrary vertices u_i and w_j. Let u_r be the vertex in G such that u_r is adjacent to w_j. Since G is connected, G has a $u_r - u_i$ path, say $u_r\ u_k\ u_{k+1}\ ....\ u_i$. Hence $w_j\ u_r\ u_k\ .....u_i$ is a $w_j - u_i$ path in $DS(G)$.

Consider the arbitrary vertices w_i and w_j. Let u_r and u_m be the vertices of G such that u_r is adjacent to w_i and u_m is adjacent to w_j. Since G is connected, G has a u_r-u_m path, say $u_r\ u_{r+1}\ ....u_m$. Hence $w_i\ u_r\ u_{r+1}\ .....\ u_m\ w_j$ is a $w_i - w_j$ path in $DS(G)$.

Therefore, $\omega(G)=1 = \omega\ (DS(G))$.

Case (ii) : Let G be disconnected.

Let $G_1, G_2,.....,G_k$ $k \geq 2$ be the components of G. If G_i and G_j have vertices of same degree for some i, j let $u\in V(G_i)$ and $v\in V(G_j)$ be such that $d(u) = d(v)$. By the definition of $DS(G)$, there exist a vertex w such that w is adjacent to u and v. Hence $\omega\ (DS(G)) \leq k\text{-}1 < k = \omega(G)$.

If there is no pair i and j such that G_i and G_j have vertices of same degree, then

$\omega(DS(G)=k=\omega(G)$.

Hence $\omega\ (DS(G)) \leq \omega\ (G)$.

From case (i) of the above theorem, if G is connected, then $DS(G)$ is connected. But converse of this result is not true.

For, if $G = G_1\cup G_2\cup....\cup G_n$, where G_i is a connected r regular graph, then $DS(G)$ is connected but G is not connected.

Theorem 7 : If G is an acyclic graph with k-components, then $DS(G)$ contains atleast $2k$ vertices of degree 2.

Proof : Let $G = G_1\cup G_2\cup...\cup G_k$, where G_i $(1 \leq i \leq k)$ is a component of G. Since each G_i is a tree, there exists vertices u_i and v_j such that $d(u_i) = d(v_j) =1$.

Therefore, $d^*(u_i) = d(u_i) +1 = 2$ and $d^*(v_j)=d(v_j)+1=2(1\leq i\leq k,\ 1\leq j\leq k)$. Hence $DS(G)$ contains atleast $2k$ vertices of degree 2.

We know $DS(K_n) = K_{n+1}$, $n>1$. On the other hand, if $G\neq K_n$, then $DS(G)$ is not even regular.

Theorem 8 : Let G be a graph with p vertices and q edges.

1. If $G(\neq K_p)$ is regular, then $DS(G)$ is not regular.
2. If G is not regular with p even, then $DS(G)$ is not regular.

Proof: (i) If G be regular of degree k. Since $G \neq K_p$, $d(u) = k \neq p\text{-}1$. Therefore, $k < p\text{-}1$ ____(1). Let $V(DS(G))\backslash V(G) = \{w\}$. Since w is adjacent to all the vertices of G, $d^*(w)=p>k+1$. (by(1)). But $d^*(u)=d(u)+1 = k+1$. Hence $DS(G)$ is not regular.

(ii) Suppose G is not regular with p even. Let u and v be the vertices of G such that $d(u) \neq d(v)$.

If there is no vertex u_1 such that $d(u_1) = d(u)$ and there is no vertex v_1 such that $d(v_1) = d(v)$, then $d^*(u) = d(u)$ and $d^*(v) = d(v)$. Therefore, $d^*(u) \neq d^*(v)$.

Suppose G has vertices $u_1, u_2,\ldots u_m$ and $v_1, v_2,\ldots v_n$ such that $d(u_i)=d(u)$ and $d(v_1)=d(v)$, $m, n > 1$. Then $d^*(u)= d(u)+1$, $d^*(v) = d(v)+1$. Since $d(u) \neq d(v)$, we get $d^*(u) \neq d^*(v)$.

Suppose there is no vertex u_1 such that $d(u_1)=d(u)$ and G has vertices $v_1, v_1,\ldots v_m$ such that $d(v_i)=d(v)$.

Case (i) : $m \neq p\text{-}2$.

Then there is a vertex x whose degree is not equal to $d(u)$ and $d(v)$. If x is the only vertex of degree $d(x)$, then $d^*(x)=d(x)$. Since $d^*(u)=d(u)$, we get $d^*(x) \neq d^*(u)$, it has vertices $x_1, x_2,\ldots x_r$ such that $d(x_i)=d(x)$, then $d^*(x)=d(x)+1$

Since $d^*(v)=d(v)+1$, implies $d^*(x) \neq d^*(v)$. Hence $DS(G)$ is not regular.

Case (ii) : $m= p\text{-}2$.

Let w be the vertex in $DS(G)$ such that w is adjacent to $v, v_1,\ldots v_{m-1}, v_m$. Therefore $d^*(w)=p-2+1=p\text{-}1$.

Suppose $DS(G)$ is regular. Then $d^*(u)= d^*(v_i)=d^*(v)=d^*(w)=p\text{-}1$.

We have $d^*(u)=d(u)$ this implies $d(u)= p\text{-}1$.....(2).

Now $d^*(v)=d(v)+1$ and $d^*(v_i)=d(v_i)+1$ and hence $d(v)=p\text{-}2, d(v_i)=p\text{-}2$.

Therefore $2q = d(u) + d(v) + \sum d(v_i)$

$= (p\text{-}1) + (p\text{-}2)(p\text{-}1)$

$= (p\text{-}1)^2$ .....(3). Since p is even, $(p\text{-}1)^2$ is odd. This contradicts (3) and hence $DS(G)$ is not regular.

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On $\Im_1\Im_2$-semi pre open sets and $\Im_1\Im_2$—quasi open sets

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Abstract

$\Im_1\Im_2$–semi pre open sets are characterized by conditions. Condition under which a $\Im_1\Im_2$–semi pre open set becomes a $\Im_1\Im_2$– semi open set is given. Also we define $\Im_1\Im_2$–quasi open sets in a biological space $(X,\Im_1\Im_2)$ and discuss their properties.

(Keywords : $\Im_1\Im_2$–pre open/$\Im_1\Im_2$–semi pre open/ $\Im_1\Im_2$–regular closed/$\Im_1\Im_2$–quasi open/pair wise semi continuity)

Introduction

The concept of a semi pre-open set in a unital topological space was introduced by Andrijevic[1] in the year 1986. A study of semi pre-open set in bitopological spaces was made by Khedr *et al.* in the year 1992.

In the present note, we characterize $\Im_1\Im_2$–semi pre open sets in a bitopological space $(X\Im_1\Im_2)$. As regards notation, Cl(A) and Int(A) denote respectively the closure and the interior of a set A.

We recall

Definition 1 : (See Fakutake[3]) Let (X, $\Im_1\Im_2$) be a bitopological space. A subset A of X is said to be $\Im_1\Im_2$–semi open in X if $A \subset \Im_2$–Cl($\Im_1$ – Int(A)).

Definition 2 : A subset A in a bitopological space (X, $\Im_1\Im_2$) is said to be $\Im_1\Im_2$–regular closed in X if $A = \Im_2$ – Cl($\Im_1$-Int(A)).

Definition 3 : (See Khedr *et al*[2].) A subset U of a bitopological space (X, $\Im_1\Im_2$) is called a $\Im_1\Im_2$–pre open set in X if $U \subset \Im_1$-Int($\Im_2$–Cl(U)).

Definition 4 : (See Khedr *et al.*[2]) A subset A of a bitopolpgiacal space (X, $\Im_1\Im_2$) is said to be $\Im_1\Im_2$–semi pre open in X if there exits a $\Im_1\Im_2$–pre open set v in X such that $U \subset A \subset (\Im_2$–Cl($U$)).

Definition 5 : A subset A of a bitopological space ((X, $\Im_1\Im_2$) is $\Im_1\Im_2$–semi closed in $\Im_1$-Int($\Im_2$–Cl(A)) $\subset A$. This follows from definition 1.

We required the following preliminary results.

Lemma 1 : (See Chandrasekhra Rao[4]) For every open set G in a topological space (X,$\Im$) and for every subset S of X, Cl(S) $\cap$ $G \subset$ Cl($S \cap G$).

Lemma 2 : Let $(X, \mathfrak{I}_1, \mathfrak{I}_2)$ be a bitopological space with $\mathfrak{I}_1 \subset \mathfrak{I}_2$ and let $A \subset X$, Then $U = A \cap [\mathfrak{I}_1\text{-Int}(\mathfrak{I}_2\text{–Cl}(A))]$ is a $\mathfrak{I}_1\mathfrak{I}_2$–pre open set.

Proof : We have $\mathfrak{I}_1\text{–Int}(\mathfrak{I}_2\text{–Cl}(U)) = \mathfrak{I}_1\text{-Int}[\mathfrak{I}_2\text{–Cl}\{A \cap \mathfrak{I}_1\text{–Int}(\mathfrak{I}_2\text{–Cl}(A))\}$

$\supset \mathfrak{I}_1\text{-Int}[\mathfrak{I}_2\text{-Cl}(A) \cap \mathfrak{I}_1\text{-Int}(\mathfrak{I}_2\text{-Cl}(A))]$

by taking $S = A$ in Lemma 1 and by noting that $\mathfrak{I}_1\text{-Int}(\mathfrak{I}_2\text{-Cl}(A)) \in \mathfrak{I}_2$ on account of the hypothesis that $\mathfrak{I}_1 \subset \mathfrak{I}_2$

Now $\mathfrak{I}_1\text{-Int}[(\mathfrak{I}_2\text{-Cl}(A) \cap \mathfrak{I}_1\text{-Int}(\mathfrak{I}_2\text{-Cl}(A))]$ $= \mathfrak{I}_1\text{-Int}\{\mathfrak{I}_1\text{-Int}((\mathfrak{I}_2\text{-Cl}(A)\}$

$= \mathfrak{I}_1\text{-Int}(\mathfrak{I}_2\text{-Cl}(A)$

$\supset [\mathfrak{I}_1\text{-Int}(\mathfrak{I}_2\text{-Cl}(A))] \cap A = U$

Therefore, $U \subset \mathfrak{I}_1\text{-Int}(\mathfrak{I}_2\text{-Cl}(A))$. Hence U is $\mathfrak{I}_1\mathfrak{I}_2$-pre open in X

Lemma 3 : Let A be $\mathfrak{I}_1\mathfrak{I}_2$–semi closed subset of a bitopological space $(X, \mathfrak{I}_1, \mathfrak{I}_2)$.

Then $\mathfrak{I}_1\text{–Int}((X_2\text{-Cl}(A)) = \mathfrak{I}_1\text{-Int}(A)$.

Proof : Since A is $\mathfrak{I}_1\mathfrak{I}_2$–semi closed, We have $\mathfrak{I}_1\text{–Int}(\mathfrak{I}_2\text{–Cl}(A)) \subset A$.

Hence $\mathfrak{I}_1\text{–Int}(\mathfrak{I}_1\text{–Int}(\mathfrak{I}_2\text{–Cl}(A))) \subset \mathfrak{I}_1\text{–Int}(A)$.

That is, $\mathfrak{I}_1\text{–Int}(\mathfrak{I}_2\text{–Cl}(A)) \subset \mathfrak{I}_1\text{–Int}(A) \subset \mathfrak{I}_1\text{–Int}(\mathfrak{I}_2\text{–Cl}(A))$.

Therefore $\mathfrak{I}_1\text{–Int}(\mathfrak{I}_2\text{–Cl}(A)) = \mathfrak{I}_1\text{–Int}(A)$.

A Characterization

Theorem 1 : Let $(X, \mathfrak{I}_1\mathfrak{I}_2)$ be a bitopological space and let A be a subset of X. Then (a)$\Rightarrow$(b)$\Rightarrow$(c); and (c) with $\mathfrak{I}_1 \subset \mathfrak{I}_2 \Rightarrow$(a), where

(a) A is $\mathfrak{I}_1\mathfrak{I}_2$-semi pre open in X.

(b) $A \subset \mathfrak{I}_2\text{-Cl}(\mathfrak{I}_1\text{–Int}(\mathfrak{I}_2\text{-Cl}(A))$.

(c) $\mathfrak{I}_2\text{-Cl}(A)$ is $\mathfrak{I}_1\mathfrak{I}_2$-regular closed in X.

Proof : *Step 1* : Suppose that (a) holds. Then there exists a $\mathfrak{I}_1\mathfrak{I}_2$-pre open set U such that

$$U \subset A \subset \mathfrak{I}_2\text{-Cl}(U). \quad (1)$$

But then

$$\mathfrak{I}_2\text{-Cl}(\mathfrak{I}_1\text{-Int}(\mathfrak{I}_2\text{-Cl}(A))) = \mathfrak{I}_2\text{-Cl}(\mathfrak{I}_1\text{-Int}(\mathfrak{I}_2\text{-Cl}(U))). \quad (2)$$

Since U is $\mathfrak{I}_1\mathfrak{I}_2$-pre open, we have $U \subset \mathfrak{I}_1\text{–Int}(\mathfrak{I}_2\text{-Cl}(U))$. This gives

$$\mathfrak{I}_2\text{-Cl}(U) \subset \mathfrak{I}_2\text{-Cl}(\mathfrak{I}_1\text{-Int}(\mathfrak{I}_2\text{-Cl}(U))). \quad (3)$$

From (2) and (3) it follows that

$$\mathfrak{I}_2\text{-Cl}(U) \subset \mathfrak{I}_2\text{-Cl}(\mathfrak{I}_1\text{–Int}(\mathfrak{I}_2\text{-Cl}(A))). \quad (4)$$

Now owing to (1) and (4) $A \subset \mathfrak{I}_2\text{-Cl}(\mathfrak{I}_1\text{-Int}(\mathfrak{I}_2\text{-Cl}(A)))$. This is (b). Thus (a) $\Rightarrow$(b).

Step 2 : Suppose that (b) holds. Then $A \subset \mathfrak{I}_2\text{-Cl}(\mathfrak{I}_1\text{–Int}(\mathfrak{I}_2\text{-Cl}(A)))$.

This gives $\mathfrak{I}_2\text{-Cl}(A) \subset \mathfrak{I}_2\text{-Cl}(\mathfrak{I}_1\text{-Int}(\mathfrak{I}_2\text{-Cl}(A)))$. (5)

But always $\mathfrak{I}_1\text{-Int}(\mathfrak{I}_2\text{-Cl}(A))) \subset \mathfrak{I}_2\text{-Cl}(A)$ and so

$$\mathfrak{I}_2\text{–Cl}(\mathfrak{I}_1\text{–Int}(\mathfrak{I}_2\text{–Cl}(A))) \subset \mathfrak{I}_2\text{ –Cl}(A) \quad (6)$$

Thus, $\mathfrak{I}_2$-Cl(A) is $\mathfrak{I}_1\mathfrak{I}_2$-regular closed, and hence (b) implies (c).

Step 3 : Suppose that (c) holds with $\mathfrak{I}_1 \subset \mathfrak{I}_2$ then

$$\mathfrak{I}_2\text{-Cl}(A)=\mathfrak{I}_2\text{-Cl}(\mathfrak{I}_1\text{-Int}(\mathfrak{I}_2\text{-Cl}(A))) \quad (7)$$

Take $U=A\cap[\mathfrak{I}_1\text{-Int}(\mathfrak{I}_2\text{-Cl}(A))]$. Then by Lemma 2 U is a $\mathfrak{I}_1\mathfrak{I}_2$-pre open set in X and $U \subset A$ by the definition of U. Furthermore

$$\mathfrak{I}_1\text{-Int}(\mathfrak{I}_2\text{-Cl}(A))=[\mathfrak{I}_2\text{-Cl}(A)]\cap[\mathfrak{I}_1\text{-Int}(\mathfrak{I}_2\text{-Cl}(A))] \quad (8)$$

But $\mathfrak{I}_1 \subset \mathfrak{I}_2$ by hypothesis and so $\mathfrak{I}_1$-Int($\mathfrak{I}_2$-Cl(A)) belongs to $\mathfrak{I}_2$.

Hence we invoke Lemma 2 with $S=\mathfrak{I}_2$-Cl(A) and obtain

$$[\mathfrak{I}_2\text{-Cl}(A)]\cap[\mathfrak{I}_1\text{-Int}(\mathfrak{I}_2\text{-Cl}(A))] \subset \mathfrak{I}_2\text{-Cl}[A\cap\{\mathfrak{I}_1\text{-Int}(\mathfrak{I}_2\text{-Cl}(A))\}] \quad (9)$$

From (8) and (9) we obtain $\mathfrak{I}_1$-Int($\mathfrak{I}_2$-Cl(A)) $\subset \mathfrak{I}_2$-Cl(U).

$$\text{Consequently } \mathfrak{I}_2\text{-Cl}(\mathfrak{I}_1\text{-Int}(\mathfrak{I}_2\text{-Cl}(A))) \subset \mathfrak{I}_2\text{-Cl}(U) \quad (10)$$

Now using (7) and (10) we get $A \subset \mathfrak{I}_2$-Cl(A) $\subset \mathfrak{I}_2$-Cl(U). We have, thus, shown that there exists a $\mathfrak{I}_1\mathfrak{I}_2$-pre open set U in X such that $U \subset A \subset \mathfrak{I}_2$-Cl($U$). This is (a).

Hence (c) with $\mathfrak{I}_1 \subset \mathfrak{I}_2$ implies (a)

Theorem 2 : Let A be a $\mathfrak{I}_1\mathfrak{I}_2$-semi pre open set in a bitopological space (X, $\mathfrak{I}_1\mathfrak{I}_2$).

If A is $\mathfrak{I}_1\mathfrak{I}_2$-semi closed, then A is $\mathfrak{I}_1\mathfrak{I}_2$-semi open

Proof : Since A is $\mathfrak{I}_1\mathfrak{I}_2$-semi pre open, $A \subset \mathfrak{I}_2$-Cl($\mathfrak{I}_1$-Int($\mathfrak{I}_2$-Cl(A))).

Since A is $\mathfrak{I}_1\mathfrak{I}_2$-semi closed, we have, by Lemma 3, that

$$\mathfrak{I}_1\text{-Int}(\mathfrak{I}_2\text{-Cl}(A))= \mathfrak{I}_1\text{-Int}(A).$$

Therefore $A \subset \mathfrak{I}_2$-Cl($\mathfrak{I}_1$-Int(A)) and so A is semi open.

The following example shows that $\mathfrak{I}_1\mathfrak{I}_2$-semi closedness of A in Theorem 2 cannot be dropped.

Example 1 : Let X be the set {0,1} endowed with $\mathfrak{I}_1=\{X, \phi, \{0\}\}$ and $\mathfrak{I}_2=\{X, \phi\{1\}\}$

Take $A=\{1\}$. Then $\mathfrak{I}_2$-Cl($\mathfrak{I}_1$-Int($\mathfrak{I}_2$-Cl(A)))=X which contains A.

Therefore A is $\mathfrak{I}_1\mathfrak{I}_2$-semi pre open set in ($X$, $\mathfrak{I}_1$,$\mathfrak{I}_2$).

But $\mathfrak{I}_2$-Cl($\mathfrak{I}_1$-Int(A))=ϕ and so A is not contained in $\mathfrak{I}_2$-Cl($\mathfrak{I}_1$-Int(A)).

This shows that A is not $\mathfrak{I}_1\mathfrak{I}_2$-semi open

Also $\mathfrak{I}_1$-Int($\mathfrak{I}_2$-Cl(A))=$X \not\subset A$, showing that A is not $\mathfrak{I}_1\mathfrak{I}_2$-semi closed.

Theorem 3 : (See Fukutake[3]) Let A be a subset of a bitopological space (X, $\mathfrak{I}_1\mathfrak{I}_2$).

Then A is $\mathfrak{I}_1\mathfrak{I}_2$-semi open if and only if there exists a $\mathfrak{I}_1$-open set O such that

$$O \subset A \subset \mathfrak{I}_1\text{-Cl}(O).$$

Here we prove an analogous result :

Theorem 4 : Let A be a subset of a bitopological space (X, $\mathfrak{I}_1$,$\mathfrak{I}_2$). Then A is $\mathfrak{I}_1\mathfrak{I}_2$-semi open if and only if there exists a $\mathfrak{I}_1\mathfrak{I}_2$-semi open set U such that

$U \subset A \subset \mathfrak{I}_2\text{-Cl}(U)$.

Proof: If A is $\mathfrak{I}_1\mathfrak{I}_2$-semi open, we take $U = A$ so that we have $U = A \subset A \subset \mathfrak{I}_2\text{-Cl}(A)$

$$= \mathfrak{I}_2\text{-Cl}(U)$$

On the other hand, if there exists $\mathfrak{I}_1\mathfrak{I}_2$-semi open set U such that

$U \subset A \subset \mathfrak{I}_2\text{-Cl}(U)$,

then $U \subset \mathfrak{I}_2\text{-Cl}(\mathfrak{I}_1\text{-Int}(U)$.

and accordingly

$\mathfrak{I}_1\text{-Int}(U) \subset U \subset A \subset \mathfrak{I}_2\text{-Cl}(\mathfrak{I}_2\text{-Cl}(\mathfrak{I}_1\text{-Int}(U)))$.

Take $V = \mathfrak{I}_1\text{-Int}(U)$, Then V is a $\mathfrak{I}_1$-open set with $V \subset A \subset \mathfrak{I}_2\text{-Cl}(V)$.

Hence, by the Fukutake Theorem quoted above, A is $\mathfrak{I}_1\mathfrak{I}_2$-semi open.

A New Class of Sets

Motivated by Theorem 4, we have the following definition :

Definition 6 : Let A be a subset of a bitopological space $(X, \mathfrak{I}_1, \mathfrak{I}_2)$. Then A is called $\mathfrak{I}_1\mathfrak{I}_2$-quasi open set if there exists a $\mathfrak{I}_1\mathfrak{I}_2$-semi open set U such that

$$U \subset A \subset \mathfrak{I}_1\text{-Cl}(U)$$

Now we prove a series of results concerning the properties of $\mathfrak{I}_1\mathfrak{I}_2$-quasi open set

Theorem 5 : If A is a $\mathfrak{I}_1\mathfrak{I}_2$-semi open set, then A is $\mathfrak{I}_1\mathfrak{I}_2$-quasi open

Proof : Suppose that A is $\mathfrak{I}_1\mathfrak{I}_2$-semi open. Noting that $A \subset A \subset \mathfrak{I}_1\text{-Cl}(A)$ and taking $U = A$, it follows that $U \subset A \subset \mathfrak{I}_1\text{-Cl}(U)$.

Hence A is $\mathfrak{I}_1\mathfrak{I}_2$-quasi open.

Remark 1 : The following example shows that the converse of the above theorem is not true.

Consider $X = \{a,b,c,\}$. Let $\mathfrak{I}_1 = \{\phi, X, \{a\}, \{b\}, \{a,b\}\}$ and $\mathfrak{I}_2 = \{\phi, X, \{a\}, \{b\}, \{a,b\}, .\{a,c\}\}$ be topologies on X. Take $A = \{b,c\}$. Then A is a $\mathfrak{I}_1\mathfrak{I}_2$-quasi open set which is not $\mathfrak{I}_1\mathfrak{I}_2$-semi open.

Theorem 6 : Let $f : (X, \mathfrak{I}_1\mathfrak{I}_2) \to (Y, \sigma_1, \sigma_2)$ be a pairwise continuous and pairwise open function. If A is $\mathfrak{I}_1\mathfrak{I}_2$-semi open, then $f(A)$ is $\sigma_1\sigma_2$-semi open. (See Theorem 4.1 in Khedr *et al.*[2])

Theorem 7 : Let $f : (X, \mathfrak{I}_1, \mathfrak{I}_2) \to (Y, \sigma_1, \sigma_2)$ be a pairwise open function. If A is $\mathfrak{I}_1\mathfrak{I}_2$-quasi open, then $f(A)$, is $\sigma_1\sigma_2$-quasi open.

Proof : Since A is quasi open, there exists a $\mathfrak{I}_1\mathfrak{I}_2$-semi open set U such that

$U \subset A \subset \mathfrak{I}_1\text{-Cl}(U)$.

But then $f(U) \subset f(A) \subset f(\mathfrak{I}_1\text{-Cl}(U))$.

Since f is pair wise continuous,

$$f(\mathfrak{I}_1\text{-Cl}(U)) \subset \sigma_1\text{-Cl}(f(U)).$$

Therefore $f(U)) \subset f(A) \subset \sigma_1$ - $\text{Cl}(f(U))$.

Also, by theorem, 6 $f(U)$ is $\sigma_1\sigma_2$-semi open. So, $f(A)$ is $\sigma_1\sigma_2$-quasi open

Theorem 8 : Let $f : (X, \mathfrak{I}_1, \mathfrak{I}_2) \to (Y, \sigma_1, \sigma_2)$ be pairwise continuous and pairwise open function. If V is a $\sigma_1\sigma_2$-semi

open set in Y, then $f^1(V)$ is $\mathfrak{I}_1\mathfrak{I}_2$-semi open in X.

Proof: Since V is a $\sigma_1\sigma_2$-semi open set, there exists a σ_1-open set W such that

$$W \subset V \subset \sigma_2\text{-Cl}(W).$$

But then

$$f^{-1}(W) \subset f^{-1}(V) \subset f^{-1}(\sigma_2\text{-Cl}(W)).$$

Since f is pairwise open, we have

$$f^1(\sigma_2\text{-Cl}(W)) \subset \mathfrak{I}_2\text{-Cl}(f^{-1}(W)).$$

and so $f^{-1}(W) \subset f^{-1}(V) \subset \mathfrak{I}_2\text{-Cl}(f^{-1}(W))$

Pairwise semi continuity of f shows that $f^{-1}(W)$ is a $\mathfrak{I}_1\mathfrak{I}_2$-semi open set in X.

Hence, by Theorem 1, it follows that $f^{-1}(V)$ is a $\mathfrak{I}_1\mathfrak{I}_2$-semi open set in X.

Theorem 9 : let $f : (X_1, \mathfrak{I}_1,\mathfrak{I}_2) \to (Y, \sigma_1\sigma_2)$ be a pairwise semi continuous and pairwise open function. If V is a $\sigma_1\sigma_2$-quasi open set in Y, then $f^{-1}(V)$ is a $\mathfrak{I}_1\mathfrak{I}_2$-quasi open set in X

Proof: It is given that V is a $\sigma_1\sigma_2$-quasi open set in Y. Hence there exist a $\sigma_1\sigma_2$-semi open set W such that

$$W \subset V \subset \sigma_1\text{-Cl}(W).$$

Whence

$$f^{-1}(W) \subset f^{-1}(V) \subset f^{-1}(\sigma_1\text{-Cl}(W)).$$

Since f is pairwise open function, we have

$$f^{-1}(\sigma_1\text{-Cl}(W)) \subset \mathfrak{I}_1\text{-Cl}(f^{-1}(W)).$$

Therefore, it follows that

$$f^{-1}(W) \subset f^{-1}(V) \subset \mathfrak{I}_1\text{-Cl}(f^{-1}(W))$$

Also, by Theorem 8, $f^{-1}(W)$ is a $\mathfrak{I}_1\mathfrak{I}_2$-semi open set. Therefore $f^{-1}(V)$ is a $\mathfrak{I}_1\mathfrak{I}_2$-quasi open set in $(X, \mathfrak{I}_1,\mathfrak{I}_2)$.

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